TROPHIC ECOLOGY OF NEARSHORE FISHES IN GLACIALLY-INFLUENCED ESTUARIES OF SOUTHEAST ALASKA

By

Emily Jean Whitney, B.S.

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of

Master of Science

in

Fisheries

University of Alaska Fairbanks

August 2016

APPROVED:

Anne H. Beaudreau, Committee Chair
Carolyn A. Bergstrom, Committee Member
Emily R. Howe, Committee Member
Franz J. Mueter, Chair
Graduate Program in Fisheries
S. Bradley Moran, Dean
School of Fisheries and Ocean Sciences
Michael Castellini,
Dean of the Graduate School

Abstract

Estuaries in Southeast Alaska (SEAK) are linked to terrestrial ecosystems by the flow of freshwater from plentiful precipitation and glacial runoff. This thesis examined the trophic ecology of nearshore fishes in SEAK estuaries to advance our understanding of how deglaciation and resulting shifts in the timing and magnitude of freshwater runoff will affect estuarine food webs. The goals of this work were to characterize seasonal variation in the feeding ecology of an abundant estuarine predator across three glacially-influenced sites and to examine the relative contribution of organic matter (OM) from terrestrial-riverine sources to the diets of estuarine consumers. In chapter one, stomach contents of Pacific staghorn sculpin (*Leptocottus armatus*) were analyzed to test the hypothesis that diets would differ across sampling sites and months, reflecting variation in freshwater runoff and the phenology of estuarine organisms. Stomach contents of staghorn sculpins were collected monthly between April and September 2014, from intertidal sites at mouths of rivers that differ in their headwater hydrology. Staghorn sculpins consumed a variety of prey, including gammarids, mysids, isopods, polychaetes, and other freshwater-tolerant prey, as well as juvenile fish. Weak to moderate differences observed in diet composition across sites and months likely reflected spatial and seasonal shifts in the occurrence of freshwater-tolerant invertebrates and young-of-the-year fishes. Overall, the ability of staghorn sculpins to take advantage of a variety of prey across variable conditions may make them resilient to environmental change. In chapter two, I examined trophic linkages between terrestrial and marine food webs by using stable isotope analysis to evaluate the relative contribution of terrestrial-riverine OM to the diets of estuarine consumers. Analyses showed limited use of terrestrial-riverine OM by marine fishes (*Leptocottus armatus* and *Platichthys stellatus*) and more variable use by anadromous fishes (*Salvelinus malma* and *Oncorhynchus kisutch*).

Intertidal invertebrates used more terrestrial-riverine OM than fish, with greater use of allochthonous OM earlier in the summer. Despite the documented availability of terrestrial-riverine OM, estuarine consumers showed limited use of this resource. These findings inform our baseline understanding of trophic linkages in glacially-influenced estuaries, a critical first step in evaluating future climate driven changes to coastal ecosystems.

Table of Contents

List of Figures

Chapter 1 Page

List of Tables

List of Appendices

Page

Acknowledgements

I would like to thank my advisor, Dr. Anne Beaudreau for her support and guidance throughout this project. My committee members Dr. Carolyn Bergstrom and Dr. Emily Howe contributed thoughtful advice and direction to the project. Carolyn Bergstrom especially shared her knowledge of sampling techniques and feeding ecology. Emily Howe contributed her knowledge of stable isotope ecology and estuary dynamics. Thank you all for the many rounds of twenty questions! I appreciated the way my committee worked with me through the process and provided encouragement and energy for the project.

This project would not have possible without the support of many field volunteers. Thank you especially to Asia Beder, Ragnhildur Fridriksdottir, and Melissa Rhodes-Reese for your willingness to wake early and deal with staghorn sculpin spines on many occasions. Doug Duncan provided invaluable support both in the field and in the lab. Thank you to Jason Fellman at the University of Alaska Southeast for sharing river data and water quality sampling equipment.

Thank you to members of the Beaudreau lab and students, past and present, in the University of Alaska Fairbanks School of Fisheries and Ocean Sciences for your support and the ways you shared your skills and stretched me to learn about other areas of research. I would like to thank my family and friends for their wholehearted support and encouragement to pursue education and knowledge. I could not have done it without you.

I would also like to acknowledge my funding sources for their role in making this research possible: Alaska EPSCoR, Alaska Sea Grant, and the University of Alaska Fairbanks.

General Introduction

Each year approximately 849 km^3 of freshwater enter the Gulf of Alaska (GOA), a volume roughly 1.5 times greater than the Mississippi River discharge into the Gulf of Mexico (Hill et al. 2015). This freshwater mixes with marine waters in estuaries, which are dynamic areas home to a diversity of ecologically and economically important species (Arimitsu et al. 2003; Baker et al. 2011). Freshwater runoff affects the physical and chemical environment of estuaries (e.g., temperature, turbidity, salinity, and nutrient transport; Sklar and Browder 1998, Hood and Berner 2009, Fellman et al. 2010). In turn, the structure and dynamics of biological communities are also affected, including the flow of nutrients and organic matter (OM) from terrestrial habitats to marine food webs (Sklar and Browder 1998; Kostecki et al. 2010; Vinagre et al. 2011). For its area, Southeast Alaska (SEAK) contributes disproportionately to the freshwater runoff into the GOA (Neal et al. 2010). Within SEAK, nearly 30% of the runoff originates from glacial sources (Neal et al. 2010) due to the region's high rate of glacial turnover, the annual cycle of melting and accumulation (Arendt et al. 2002).

There is a need to understand the trophic ecology of estuarine consumers in glacially-influenced coastal ecosystems of SEAK given future climate conditions that will alter the structure, function, and productivity of these systems (Hood and Berner 2009; O'Neel et al. 2015). Already, Alaskan ecosystems are changing in response to the thinning and retreating of glaciers in over 95% of glaciated areas (Larsen et al. 2007). These changes will likely be amplified by the increased temperatures and precipitation combined with reduced snowfall predicted for SEAK over the next 100 years (SNAP 2008, McAfee et al. 2014). The volume of water entering the GOA will likely increase for a time and be followed by a decrease in discharge due to glacial volume loss (Hill et al. 2015). There will also be shifts in the timing of

peak runoff from forested and glaciated areas, resulting in changes to estuary habitats. An understanding of the current structure and dynamics of glacially-influenced estuaries is needed to evaluate these future impacts.

Estuaries of SEAK represent a class of high-latitude estuaries that are understudied compared to estuaries in lower latitudes that are significantly influenced by human activities, such as Puget Sound, San Francisco Bay, and Chesapeake Bay in the United States. The 12-volume Treatise on Estuarine and Coastal Science (Wolanski and McLusky 2011) is one of the most up-to-date references on estuarine and coastal science and management. In the Treatise, an entire volume is devoted to research on systems affected by development and other human activities (Volume 8; Wolanski and McLusky 2011), but there are only limited references to the ecology of coastal systems currently affected by glacial ice (but see Forbes 2011). Not only are SEAK's estuaries largely undeveloped and subject to rapid climate change due to melting glaciers (Larsen et al. 2007), but the proximity of glacial headwaters to the coast results in short transit times of freshwater and OM to estuaries. The average length of streams with glacial headwaters in SEAK is just 10 km, two orders of magnitude shorter than the average for the Western United States (O'Neel et al. 2015). These aspects set SEAK estuaries apart from other longer, non-glacially-influenced estuaries.

This thesis examined the trophic ecology of nearshore fishes in SEAK estuaries to contribute to our understanding of how changes in freshwater runoff affect estuary food webs. The overarching goals of this work were to characterize seasonal variation in the feeding ecology of an abundant estuarine predator across three sites that differ in their degree of glacial influence and to examine the relative contribution of OM from terrestrial-riverine sources to the diets of estuarine consumers. In chapter one, the diets of Pacific staghorn sculpin (*Leptocottus armatus*)

were examined across three sites and six sampling months in 2014. Staghorn sculpins are abundant estuarine predators that are widely distributed along the West Coast of North America (Mecklenburg et al. 2002). To understand how the diets of staghorn sculpin changed across a period of variable freshwater inputs to the estuary, we (1) described the taxonomic composition and size of prey in staghorn sculpin diets, including the contribution of freshwater-tolerant species; and (2) examined seasonal and spatial variation in their diets across three SEAK estuaries. We used diet composition metrics and multivariate analyses to address the hypothesis that staghorn sculpin diet would differ across sampling sites and months, reflecting variation in freshwater input and the phenology of estuarine organisms.

The second chapter used stable isotope analyses to examine the contribution of allochthonous OM delivered by freshwater to the diets of estuarine consumers across multiple trophic levels. The flow of water between terrestrial and marine ecosystems is a principal mechanism for the delivery of allochthonous subsidies (Polis et al. 1997). Forecasted climate conditions (SNAP 2008, McAfee et al. 2014) will alter the hydrology of these systems and in turn, effect the delivery of allochthonous subsidies to estuarine consumers. The goal of this chapter was to characterize cross-habitat trophic linkages under current climate conditions. Specifically, the objectives were to (1) evaluate the relative contributions of terrestrial/riverine OM and marine OM to the diets of select estuarine fishes and invertebrates; and (2) examine spatial and temporal variation in OM use across three SEAK estuaries and six sampling months. We measured carbon, nitrogen, and sulfur stable isotopes in tissues of estuarine consumers and OM sources to address the hypothesis that allochthonous OM from terrestrial and riverine habitats is an important subsidy to estuarine consumers.

Chapter 1: Spatial and temporal variation in the diets of Pacific staghorn sculpin related to hydrological factors in glacially-influenced estuaries[1]

Abstract

In Southeast Alaska, a large volume of freshwater flows into estuaries from melting and retreating glaciers and high rainfall in the surrounding coastal temperate rainforest. Freshwater runoff to the coast affects the physical and chemical properties of estuarine habitats and, in turn, the structure and dynamics of biological communities. This study quantified temporal and spatial variation in the diets of Pacific staghorn sculpin (*Leptocottus armatus*) as an important step towards understanding how variation in freshwater runoff may translate into changes in the feeding ecology of a highly abundant consumer in estuaries along the west coast of North America. Stomach contents of staghorn sculpins were collected monthly (April-September 2014) from three intertidal sites at the mouths of rivers that differ in their headwater hydrology. Sampled staghorn sculpins consumed a variety of marine prey items, including epibenthic invertebrates (predominantly gammarids, mysids, isopods, and polychaetes) and juvenile fishes (Salmonidae, Cottidae, and Pleuronectidae). Prey items also included species from adjacent freshwater and terrestrial habitats. Staghorn sculpin diets showed weak to moderate differences in diet composition across sampling months and sites that differed in the magnitude and type of freshwater runoff. Diet differences reflected seasonal shifts in the contributions of freshwater-tolerant invertebrates and young-of-the-year fishes. The greatest site differences were observed between Cowee Creek estuary and Mendenhall River estuary, the watersheds with the lowest and highest glacial coverage, respectively. Despite compositional differences, four to five dominant prey items were prominent in the diet of staghorn sculpin across sites and months. Overall, the consumption of prey items that are well adapted to brackish and freshwater conditions and the

[1] Whitney, E. J., A. H. Beaudreau, D. H. Duncan. 2016. Prepared for submission to Estuaries and Coasts.

generalist feeding behavior of staghorn sculpin across variable hydrological conditions suggests that these fish may be resilient to changes in estuarine conditions.

Introduction

Each year approximately 849 km^3 of freshwater enter the Gulf of Alaska (GOA), with glaciers alone contributing a staggering 75 Gt of water to the marine environment (Hill et al. 2015; Larsen et al. 2015). The massive volume of water flowing into the GOA drives ocean circulation and patterns of primary production (Royer et al. 2001; Stabeno et al. 2004). Southeast Alaska (SEAK) contributes disproportionately to this freshwater runoff (Neal et al. 2010), covering 37% of the watershed area draining into the GOA but generating 43% of the runoff. SEAK runoff consists of precipitation and meltwater due to a high rate of glacial turnover and retreat (Arendt et al. 2002). Much of this water enters the marine environment from small coastal drainages via estuaries (Neal et al. 2010). The delivery of freshwater affects the physical and chemical environment of recipient waters (e.g., temperature, turbidity, salinity, and nutrient transport; Sklar and Browder 1998, Hood and Berner 2009, Fellman et al. 2010) and, in turn, the structure and dynamics of biological communities (Sklar and Browder 1998, Kostecki et al. 2010, Vinagre et al. 2011).

Freshwater discharge can have multifaceted impacts on estuarine food web dynamics. River flow transports nutrients and organic matter from terrestrial and freshwater habitats that are consumed by nearshore marine organisms (e.g., Howe and Simenstad 2011) and hydrological shifts affect the amount of terrestrial material available to consumers (Abrantes et al. 2013). Ecological effects of shifts in runoff may vary in relationship to the type of watershed draining into the estuary. In SEAK, there is variation in the timing and peaks of the seasonal hydrograph

across a diversity of watershed types, from heavily glaciated to rain-fed drainages (Milner and Petts 1994). Impacts of freshwater on the physical and chemical environment of estuaries, including changes in temperature and salinity, may affect the physiology of estuary organisms and lead to shifts in the spatial and temporal availability of prey to predators (Durant et al. 2007). These shifts in the feeding environment of estuarine species could alter growth and survival of early life stages of economically and ecologically important fishes. For example, synchronous declines in marine survival of coho salmon along the West Coast after 1989 were associated with increased discharge from the Fraser River in April (Beamish et al. 2000). Large increases in precipitation and glacier mass loss projected for SEAK in the coming decades (Hood and Berner 2009; McAfee et al. 2014) will affect estuary habitats and species, but there is little understanding of the downstream effects on the feeding ecology of estuarine consumers. Characterizing the current range of spatial and temporal variation in the feeding ecology of estuarine fishes is important for detecting future change.

Our study takes a first step towards understanding how seasonal variation in freshwater runoff may translate into shifts in the feeding ecology of estuarine fishes by characterizing the diets of a highly abundant, generalist consumer in SEAK estuaries. Generalist consumers, including many estuarine fishes, may provide an indirect approach to characterizing the types and sources of prey that are present in their local environment. For example, an annual index of juvenile rockfish relative abundance developed from seabird diets was found to capture the variability in rockfish abundance observed in trawl surveys (Mills et al. 2007). Pacific halibut stomach content sampling has also been used to assess the occurrence of forage fishes (Roseneau and Byrd 1997). Similarly, the opportunistic feeding behavior of Atlantic cod was found to reflect changes to benthic prey availability across time and space (Link and Garrison 2002).

Here, we focused on Pacific staghorn sculpin (*Leptocottus armatus*; hereafter referred to as staghorn sculpin) as a sampler of estuarine habitats in SEAK. They are among the most common shallow water marine sculpins along the Pacific coast (Herald 1961) and are found in high densities in estuaries and lower reaches of coastal streams, from the Gulf of Alaska to Baja California (Mecklenburg et al. 2002). Staghorn sculpins are tolerant of a wide range of temperatures and salinities (e.g., 10-30^{0}C and 12-37‰, respectively, based on laboratory studies of southern range limitations; Morris 1960). These sculpins are well adapted to dynamic estuarine conditions and consume a wide diversity of prey from across terrestrial, freshwater, and marine habitats, such as the terrestrial and aquatic life stages of insects and epibenthic marine organisms (Tasto 1975; Mace 1983). Further, their diets may fluctuate seasonally, based on prey abundance and availability (Mace 1983; Armstrong et al. 1995).

To better understand how the feeding environment of staghorn sculpins may vary with freshwater inputs to the estuary, we (1) described the taxonomic composition and size of prey in staghorn sculpin diets, including the contribution of freshwater-tolerant species; and (2) examined seasonal variation in their diets across three SEAK estuary sites downstream of rivers that differ in their headwater hydrology. We addressed the following hypotheses: (H1) staghorn sculpin diet composition will differ across months and sites, reflecting variation in the flow of freshwater into the estuaries and seasonal availability of prey. Specifically, we expected that temporal shifts in diets will reflect the phenology of estuarine species; for example, juvenile Pacific salmon (*Oncorhynchus* spp.) are present in high densities during May and June (Johnson et al. 2005), which may be reflected in staghorn sculpin diets; (H2) the greatest temporal variation in diet will occur at the most glacially-influenced site due to higher intra-annual flow fluctuations compared to estuaries fed by watersheds with lower glacial coverage; and (H3)

between-site differences will be most pronounced during months with the largest difference in river discharge among sites.

Methods

Study Area

Samples were collected from three estuary sites located near Juneau, Alaska, fed by clearwater and proglacial streams: Cowee Creek estuary (58.68158 °N, -134.951 °W), Eagle River estuary (58.53881 °N, -134.85 °W), and Mendenhall River estuary (58.32817 °N, -134.607 °W; Fig. 1.1, Table 1.1). The Mendenhall River watershed is characterized by the highest degree of glacial cover (63%) and is representative of a proglacial (glacial-fed) stream type, while the smaller Cowee Creek watershed has just 13% glacier cover and more closely represents a clearwater (precipitation-fed) stream type (Fellman et al. 2014). Intermediate to these sites is the Eagle River estuary, which is fed by the confluence of the Eagle and Herbert Rivers. The Herbert and Eagle River watersheds share a similar degree of glacier and forest cover and have a moderate degree of glacial coverage (48-49%; Fellman et al. 2014). Each of the three estuary sites is impacted by upstream changes in precipitation and increased freshwater discharge from melting glaciers. Within each estuary, samples were collected in proximity to the river mouth. All sites had shallow sloping intertidal areas with similar fine-grained (sand and mud) sediment with interspersed cobble.

Field methodology

Sampling occurred monthly from April to September 2014. Sampling months were selected to capture the period of greatest change in the freshwater hydrographs of the rivers that

drain into our selected estuaries and the peak period of marine productivity. Pilot sampling occurred in August and September 2013 and informed our 2014 sampling methodology. We collected staghorn sculpin and other estuary fishes using a beach seine (15 m x 2.4 m net with 6.3 mm square mesh) deployed from the beach by two researchers who towed the net parallel to the shoreline for 4-6 minutes before closing off the net and pulling the seine to shore. At each site, a minimum of three seine hauls were performed during daylight hours and within two hours of negative low tides (< 0 m below MLLW). All fish and macroinvertebrates were identified and counted, and length measurements taken for the first 50 individuals per seine haul. During each sampling event, water temperature, salinity, and dissolved oxygen were recorded at three randomly selected stations off the beach at 1 meter depth (YSI Pro2030). Turbidity was measured for water samples collected at the three stations (2100P TurbidiMeter). Daily discharge data ($ft^3\ s^{-1}$) were obtained when available during the sampling period (Fig. 1.2). Discharge values for the Mendenhall River were acquired from the U.S. Geological Survey (USGS, site number 15052500). Herbert River and Cowee Creek discharge values were acquired from Jason Fellman at the University of Alaska Southeast. As Eagle River discharge was not gauged, we used discharge data from the Herbert River, which flows into the Eagle River less than 1 km upstream of the river mouth. Although, the Herbert River data cannot be used as a measure of total discharge into the estuary, they do represent the variability and timing of peak flow into the estuary (Jason Fellman, University of Alaska Southeast, personal communication 3/1/2016).

Across all sampling events, 402 staghorn sculpins were collected for diet analysis [mean (SD): 168 (48) mm TL, Table 1.2]. We selected a sample size of 25 individuals to characterize the taxonomic diversity of prey in the diets based on previous studies of staghorn sculpin food habits (Armstrong et al. 1995; West et al. 2003) and data collected during pilot sampling in

September 2013. We preferentially retained the dominant size class of staghorn sculpin caught in beach seines (175 – 200 mm; Fig. 1.3). To meet the target sample size of 25 individuals/sampling event, however, we retained some larger or small fish. In some instances, catch rates were too low to meet the target sample size (Table 1.2). Staghorn sculpins were euthanized in the field and put on ice until they could be processed in the lab (typically within 4 hours).

Laboratory methodology

In the laboratory, staghorn sculpin were weighed and stomachs were removed and placed in 80% ethanol for preservation. Stomach content analysis was performed following standard methods in fish feeding ecology (Beaudreau and Essington 2007; Pasquaud et al. 2008), briefly described here. The total weight (blotted wet weight, to the nearest 0.01 g) of the contents of each stomach was recorded and then individual prey items were sorted. Prey items were identified to the lowest taxonomic resolution possible using fish and invertebrate identification keys (Butler 1980, Kozloff 1996, O'Clair and O'Clair 1998, Mecklenburg et al. 2002, Johnson et al. 2015). For each prey item, we measured blotted wet weight (to the nearest 0.01 g) and length (to the nearest 0.1 mm) for intact specimens.

Data Analysis

We used two standard metrics to characterize prey diversity and diet composition of staghorn sculpins: frequency of prey occurrence and percent composition by weight (Chipps and Garvey 2007), which were calculated for each sampling event (unique site and month combination) and overall for all sampling events. Frequency of occurrence (FO) was calculated

as the number of sampled stomachs containing a given prey taxon, divided by the total number of sampled stomachs. We calculated the FO of prey to identify common prey in staghorn sculpin diets and to quantify the frequency with which freshwater-tolerant species occurred in diets. The proportion by weight (%W) was calculated as the weight of a given prey taxon divided by the total weight of all prey in staghorn sculpin stomachs. The %W was used to characterize the diet composition of staghorn sculpins and was used as an input for the multivariate analyses described below. Prey items that were unidentifiable due to an advanced stage of digestion were excluded from the analysis, as were rocks and vegetation.

We next compared diets of staghorn sculpin across sampling months and sites using multivariate analyses. Data on prey composition by weight (%W) in each predator stomach were standardized and square root transformed, then used to construct a Bray-Curtis similarity matrix (Clarke 1993). We performed analyses of similarity (ANOSIM) on the Bray-Curtis matrix to evaluate diet differences among sites and months. ANOSIM produces an R statistic, which indicates the degree of separation between groups; R values range from 0, indicating identical samples, to 1, indicating that the samples are completely different (Clarke et al. 2014). We interpreted R values ≤ 0.25 to indicate weak differences, R values >0.26 and ≤ 0.50 reflected moderate differences, and R values >0.51 reflected strong differences between groups (Creque and Czesny 2012). ANOSIM also produces a p-value, however, the value is strongly affected by sample sizes so we did not rely on p-values for inference (Clarke et al. 2014). We used a similarity percentages (SIMPER) test to determine which prey types contributed most to differences in staghorn sculpin diets between sampling sites and months, as identified by the ANOSIM. Analyses were conducted in PRIMER V7.0, a program designed for the analysis of

ecological and environmental data (Plymouth Marine Laboratories, UK, Clarke and Gorley 2015).

We used a two-way ANOSIM to examine overall differences in diet composition across sites and months, to address our hypothesis that staghorn sculpin diet composition would differ across months and sites relating to changes in freshwater discharge and seasonal prey availability (H1). Patterns in %FO and %W were examined to address the hypothesis that temporal shifts in diets would reflect the phenology of estuarine species. To examine patterns in consumption of individual prey items corresponding to changes in freshwater discharge, the relative prey weight was calculated to address qualitatively the hypothesis that prey consumption would correspond to changes in freshwater discharge. The relative prey weight is the mean weight (g) of the prey taxa in a predator's stomach divided by predator mass (g) raised to the power of 0.75, to account for allometric shifts in metabolic rate of the predator (Essington et al. 2001; Beaudreau and Essington 2007). We examined patterns in the relative prey weight of species identified by SIMPER as contributing to differences.

We used separate one-way ANOSIM, performed on the Bray-Curtis matrix, to examine among-month differences in diets from staghorn sculpin collected within each site, and among-site differences in diets collected in each month. Among-month differences were examined to address the hypothesis that the Mendenhall River estuary would show greater temporal variation compared to the Cowee Creek and Eagle River estuaries because of higher intra-annual fluctuations in freshwater discharge at the Mendenhall site (H2). Differences among sites within months were examined to see if the diet composition of staghorn sculpins differed more strongly when the relative difference in discharge among sites was maximized (H3). Specifically, we compared diet composition of staghorn sculpin from the Cowee Creek and Mendenhall River

estuaries. These two sites were selected because if freshwater flow affects diet composition, we expected to see it when contrasting the sites with the greatest differences in freshwater flow.

Results

Site Conditions and Catch Composition

The average monthly Mendenhall River discharge was two orders of magnitude greater than that of Cowee Creek (Table 1.2), whereas Herbert River discharge was intermediate to Cowee Creek and Mendenhall River. All sites showed a peak in discharge in July and August (Fig. 1.2). For the Mendenhall River, which was gauged year round, the July and August peaks represented the highest daily total discharges and highest monthly average discharges recorded during 2014 (USGS 2014). During sampling months, river flow levels were affected by glacial runoff and snowmelt associated with summer temperatures but also high levels of precipitation. There were 61.65 cm of precipitation in June, July, and August (National Weather Service 2014), which made the summer of 2014 the wettest on record in Juneau.

At the Eagle River and Cowee Creek sites, surface water temperature increased from April to August, while at the Mendenhall River site, temperature increased from April to June and then decreased in July and August (Table 1.2), coinciding with peak discharge. The heavily glaciated Mendenhall River estuary (mean summer temperature: 9.8 ^{0}C) was cooler compared to the Eagle River estuary (mean summer temperature: 10.7 ^{0}C) and Cowee Creek estuary (mean summer temperature: 10.6 ^{0}C). Salinity generally showed the inverse relationship with temperature; salinity reached a low at all sites in July and August (Table 1.2). Turbidity at the Mendenhall River and Eagle River sites peaked in July and August, during the peak discharge window (Table 1.2). At Cowee Creek, the maximum turbidity was recorded in September.

Dissolved oxygen displayed a similar pattern across sites and declined gradually from April to September (Table 1.2).

A total of 2,411 staghorn sculpin were captured by beach seine between April and September 2014, ranging in size from 25 mm to 365 mm TL. This size range encompassed both young-of-the-year fish and mature adults, based on literature values (Tasto 1975) and observation of developed reproductive organs. Across sites, body size and abundance of staghorn sculpins were lowest in April (Fig. 1.3). Catch per unit effort (CPUE) differed significantly among sites (ANOVA, $p=0.02$) and was highest at the Mendenhall River site [mean (SD): 73.6 (52.8) staghorn sculpins/set] and lowest at Cowee Creek [5.9 (4.9) staghorn sculpins/set]. Eagle River estuary CPUE was intermediate [32.1 (36.1) staghorn sculpins/set].

Diet Composition

All staghorn sculpins retained for diet analysis (N=402) contained stomach contents. A total of 13,811 prey items from 81 taxa were identified in samples from 2014 (Table 1.3). More than 62% of the diet by weight across sampling sites and months was composed of epibenthic invertebrates, largely marine crustaceans. Gammarids (76% FO), mysids (65% FO), polychaetes (54% FO), and the isopod *Gnorimosphaeroma oregonensis* (39% FO) occurred most frequently in diets. In addition to invertebrate prey, teleost prey made up 34% of the diet by weight and occurred in 37% of the samples. Commonly occurring fish prey included juvenile Salmonidae, Cottidae, and Pleuronectidae.

Staghorn sculpins also consumed prey of terrestrial and freshwater origin. Aquatic (larvae and pupae) and terrestrial (adults) insects were found in stomachs, although at a lower frequency (8.7% FO, Table 1.3) than dominant prey items. Insects represented less than 1% by weight of

staghorn sculpin diets. Diets included freshwater larvae and pupae of aquatic flies (Diptera; <5% of identified Dipterans were adults of terrestrial origin), especially chironomid larvae, as well as adult beetles (Coleoptera) of terrestrial origin. They also consumed prey items that are found in wetland environments, such as *Americorophium spinicorne*, tube dwelling amphipods, which have been observed in high densities in nearly freshwater sloughs of the Mendenhall wetlands (Willson and Baldwin 2003). Similarly, 52% of the shrimp consumed belonged to the *Crangon* genus, a portion of which could be identified to the subspecies *Crangon franciscorum franciscorum*, which is known to inhabit estuaries with a broad range of temperatures and salinities (Butler 1980).

Staghorn sculpins consumed a range of prey sizes, from mussel (*Mytilus trossulus*) spat less than 1 mm in length to a snake prickleback (*Lumpenus sagitta*) 94 mm in length or 54% of the predator's body length. Stomach contents indicate that staghorn sculpin scavenged on prey items that were too large to fit into their mouths whole, including adult salmon carcasses and brachyuran crab legs. In addition, they demonstrated siphon snipping, where only the siphon tips of bivalves were consumed. Some staghorn sculpins ate only the exposed head and thorax of the intertidal hairy hermit crab (*Pagurus hirsutiusculus*). Despite the individual feeding behaviors captured in the diets, SIMPER analyses generally showed greater similarity in staghorn sculpin diets within a sampling site or month compared to between groups.

Seasonal and Site Trends

Staghorn sculpin diets showed weak differences among sites (two-way ANOSIM, R=0.218, p=0.001); however, the temporal differences (two-way ANOSIM, R=0.253, p=0.001) discussed above were greater than among site differences, as evidenced by the higher R-value of

0.253. Dominant prey items including gammarids, mysids, polychaetes, and isopods (*G. oregonensis*) were the largest individual contributors to differences among sites due to shifts in the percent contribution by weight across sites and sampling months (SIMPER, Table 1.5). To a lesser extent, insect prey consumption also contributed to differences among sites at a smaller scale (1.28-2.95% of differences in pairwise site comparisons, SIMPER). The relative prey weight of insects at the Mendenhall River estuary was $7.8*10^{-4}$ (SD 0.002), lower than the $2.4*10^{-3}$ (SD 0.001) at the Eagle River estuary and the $2.5*10^{-3}$ (SD 0.002) at the Cowee Creek estuary, however, differences were non-significant (ANOVA, p=0.87).

The relative prey weight of mysids, a dominant prey item in staghorn sculpin stomachs that SIMPER identified as contributing to site differences, demonstrated seasonal variation. Qualitatively, the relative prey weight of mysids in staghorn sculpins from the Mendenhall River estuary was greater from July to September compared to earlier months of lower freshwater discharge (April –June; Fig. 1.4). A moderate increase in the relative prey weight of mysids was also observed at the Eagle River site during the July-September period relative to April through June. In contrast, mysid consumption at the Cowee Creek site remained relatively stable during the same period. Both the Eagle River and Mendenhall River sites experienced a greater upswing in freshwater discharge associated with glacial meltwater in July through September compared to Cowee Creek (Fig. 1.2), which has lower glacial coverage in the watershed and lower flows. Temporal patterns in relative prey weight were also observed for the corophiid amphipod, *Americorophium spinicorne*, a freshwater tolerant species consumed by staghorn sculpin from the Mendenhall River estuary as well as from Eagle River. Consumption of this amphipod was higher in April and June, months of lower river flows, compared to July through September with higher flows (although, corophiids were absent from diets in May). Other species identified by

SIMPER as contributing to differences, such as nereid worms and *Crangon* shrimp varied in the diets (Table 1.3) but not following patterns of recorded environmental variables.

When the diet composition of staghorn sculpin was examined by site, the sites showed weak to moderate differences in diet composition across months. In support of our hypothesis, the Mendenhall River site showed more pronounced seasonal differences (one-way ANOSIM, R=0.336, p=0.001) compared to the Eagle River (one-way ANOSIM, R=0.231, p=0.001) and Cowee Creek estuaries (one-way ANOSIM, R=0.108, p=0.001). This is consistent with the fact that the Mendenhall River site experienced a greater difference in total discharge between high and low flow periods compared to the Cowee Creek site, which showed the smallest differences in total discharge among months (Fig. 1.2).

We contrasted the diet composition of staghorn sculpins from the Mendenhall River and Cowee Creek sites to address the hypothesis that differences in freshwater discharge would contribute to sites differences. In April, during low flow conditions at both sites, the Mendenhall River and Cowee Creek sites were indistinguishable by diet (one-way ANOSIM, R=0.005, p=0.46 Table 1.4). Between June and August, diet composition differed more strongly between the Mendenhall River and Cowee Creek sites (Table. 1.4). The greatest diet differences between these sites were observed in June (one-way ANOSIM, R=0.564, p=0.001) and August (one-way ANOSIM, R=0.472, p=0.001, Table. 1.4).

The strong differences in diet composition between the Mendenhall River and Cowee Creek sites in June, provided support for the hypothesis that diets would reflect site and seasonal differences in prey availability. In June, staghorn sculpin diets from all sites showed a peak in fish consumption, with up to 68% of the diet by weight attributed to fish. There were site differences in the predominant fish species consumed; for example between staghorn sculpin

diets from the Mendenhall River and Cowee Creek estuaries, multiple species of fish accounted for 28.82% of the site differences in June (SIMPER). At the Eagle River site, juvenile salmon CPUE was at its highest in June [98 (43.5) salmon/set] and these fish accounted for 69% of fish mass consumed. In contrast, at the Mendenhall River site, the June peak was attributed to predation on juvenile Pacific herring and other small fishes that could not be identified due to digestion but were likely juveniles based on length and weight data. Staghorn sculpins at the Cowee Creek site consumed predominately young-of-the-year sculpin. Juvenile fish consumption showed temporal patterns. While juvenile fish were a dominate prey item by weight in June, the proportion of fish in diets declined in July (dropping to 24.84% by weight across sites).

In August, when the difference in mean monthly discharge between Cowee Creek and Mendenhall River was maximized (Fig. 1.2), there were moderate differences in diet composition (one-way ANOSIM, $R=0.472$, $p=0.001$) between these sites. SIMPER showed that the average dissimilarity between the Cowee Creek and Mendenhall River sites peaked at 84.01% dissimilarity in August. This supports the hypothesis that site differences would be most pronounced during periods with greatest differences in discharge. Invertebrate prey in the diets of staghorn sculpins drove the differences between sites. Mysids, a prey species that showed seasonal variation, accounted for 20.82% (SIMPER) of the observed differences.

Discussion

This study examined staghorn sculpin diets in estuarine habitats receiving variable and, at times, high freshwater runoff. Staghorn sculpins consumed a variety of prey items and all sampled individuals contained prey in their stomachs. This is consistent with other studies of this

generalist predator from the U.S. west coast (Tasto 1975; Mace 1983; Armstrong et al. 1995; West et al. 2003). Diet composition showed weak to moderate differences across sampling periods with variable river flow and sites with differing watershed characteristics, as demonstrated by an analysis of similarity (ANOSIM). Despite temporal and spatial differences in diets, staghorn sculpin showed consistent use of four to five dominant prey taxa across months and sites (gammarids, mysids, polychaetes, isopods, and juvenile fish including salmon, flatfish, sculpin, and other forage fishes) in 2014 as well as in September 2013 (Fig. 1.5). As hypothesized, the greatest seasonal variation in diet was documented at the Mendenhall River, the site with the greatest seasonal range in freshwater runoff due to glacial meltwater. Differences in diets were greatest between the sites downstream of the most glaciated (Mendenhall River) and least glaciated (Cowee Creek) watersheds and were maximized in June and August. Differences in diets among months and sites may reflect temporal and spatial variation in freshwater discharge and the timing of life history events for estuarine prey (e.g., recruitment events, spawning migrations).

We found support for the hypothesis that staghorn sculpin consume prey items that are well adapted to hyposaline estuarine conditions and adjacent freshwater and terrestrial habitats. Staghorn sculpin consumed larval stages of chironomids from adjacent freshwater habitats, as well as adult life stages from the terrestrial environment. There are several potential mechanisms by which terrestrial and freshwater prey can be incorporated into staghorn sculpin diets. Terrestrial prey may fall into the water from overhanging vegetation (Nakano and Murakami 2001) and be carried downstream. Direct deposition from adjacent habitats and aeolian transport into the marine environment may also occur. It is, however, possible that staghorn sculpin make

feeding forays into adjacent habitats. For example, an individual staghorn sculpin that consumed 85 salmon eggs was more likely feeding at an upstream redd rather than intertidally.

The diets of staghorn sculpins fluctuated in time and across sites, supporting our hypothesis that diets would reflect site differences and phenology of estuarine species. The high proportion of fish in the diets of staghorn sculpins in June followed by the July decline may reflect the seasonal abundance and availability of juvenile fish. At Eagle River estuary, juvenile salmon CPUE in July [mean (SD): 1.5 (0.7) salmon/set] was a fraction of June levels [mean (SD): 98 (43.5) salmon/set] and correspondingly, juvenile salmon declined from 45%W of the diets in June to 0% in July. Further, while 2014 samples contained both salmon eggs and juvenile salmon, samples collected during pilot sampling in September 2013, also included adult salmon carcasses, coinciding with a record high pink salmon run in Southeast Alaska (Wertheimer et al. 2014). Staghorn sculpins ability to capitalize on seasonally available prey resources was shown in the apparent relationship between the fish community composition data and diet composition, particularly as related to seasonally present fish, such as salmon. However, other fish species caught in seine sets (i.e., Crescent gunnels *Pholis laeta*) showed seasonal patterns of occurrence that were not reflected in diets.

Differences between Cowee Creek and Mendenhall River in August, during a time of disparate flow, may reflect the influence of hydrological conditions on the distribution and abundance of estuarine fish and invertebrates. Mysids were the primary contributor to August differences between the Cowee Creek and Mendenhall River estuaries. The relative prey weight of mysids varied seasonally and across sites, following patterns in freshwater discharge. Mysid consumption increased at the Mendenhall River and Eagle River sites during peak flow months (July-September), while consumption at the Cowee Creek site remained stable. Mysids are found

in fresh and brackish waters, including the wetland sloughs of the Mendenhall River (Aaron Baldwin, Alaska Department of Fish and Game, personal communication 3/26/14). They have been shown to be an important component of estuarine food webs (Siegfried and Kopache 1980) and are a major food source for marine fish, mammals, and migratory birds in estuaries around the world (Kathman et al. 1986; Willson and Baldwin 2003). Based on research in other estuary and river systems, mysids are likely present year round, with both overwintering and summer generations (Johnston and Northcote 1989). Thus, it is possible that during high flow periods, mysids are flushed from upstream habitats and are more readily consumed by staghorn sculpin. The seasonal pattern in the consumption of mysids at the Mendenhall River site suggests that staghorn sculpin predation on wetland species may be affected by freshwater flow, although the life history of mysids may also be a contributing factor. There is evidence that both of these factors contribute to dietary differences, but our ability to separate the effects of freshwater flow and phenology of prey is limited by a single year of data collection.

Consistency in diet despite changing environmental conditions suggests staghorn sculpin are well adapted to cope with variability. This is supported by the body of evidence indicating estuarine flora and fauna are well adapted to life in these naturally stressed areas (Elliott and Quintino 2007). The generalist feeding behavior of staghorn sculpin, including their consistent use of dominant prey taxa across sampling periods and sites, combined with their predation on freshwater tolerant and terrestrial prey species indicate that staghorn sculpin may be resilient to environmental change associated with increased glacial meltwater.

Figures

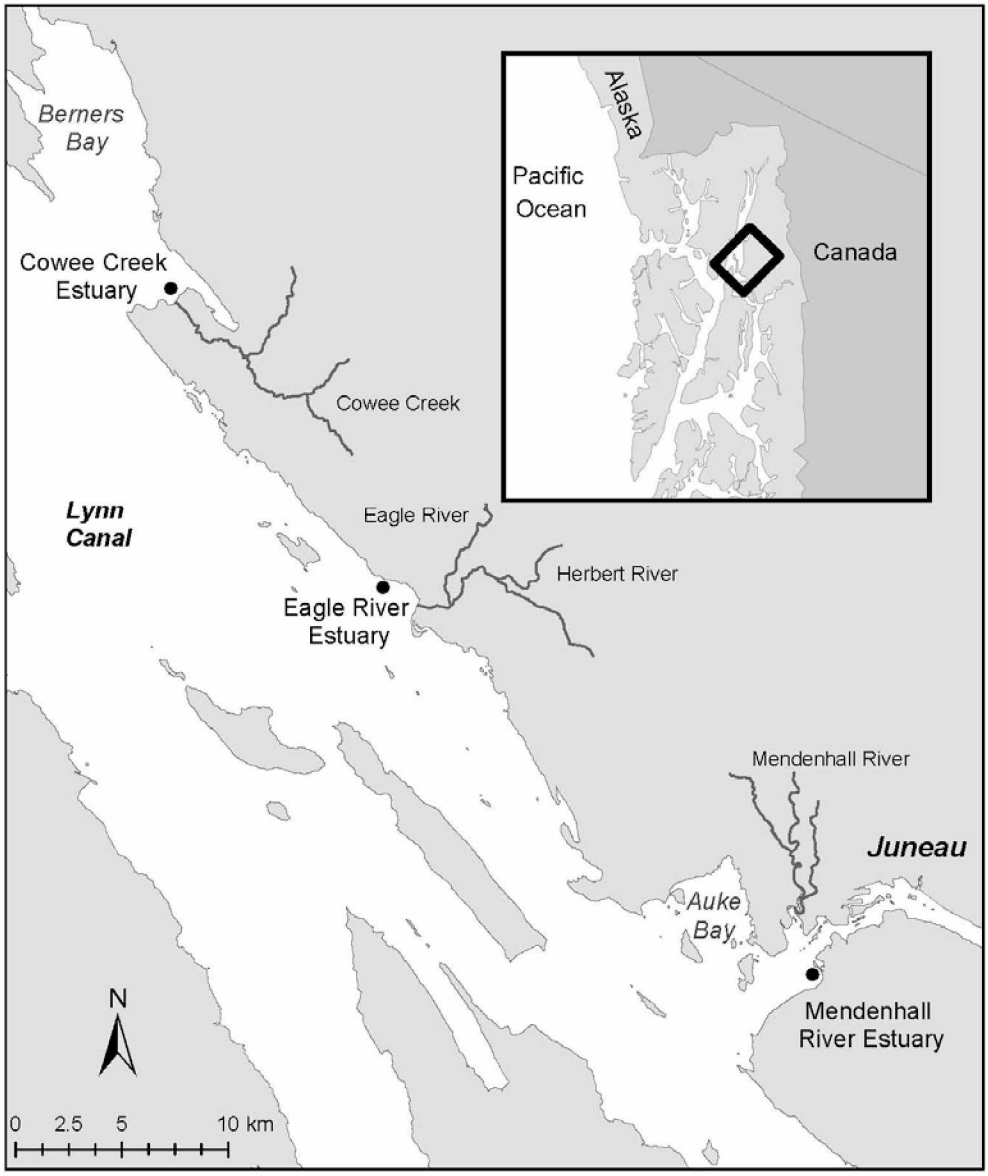


Fig. 1.1 Study site locations in Juneau, Alaska. The dominant rivers that drain into each of the estuaries are shown in black

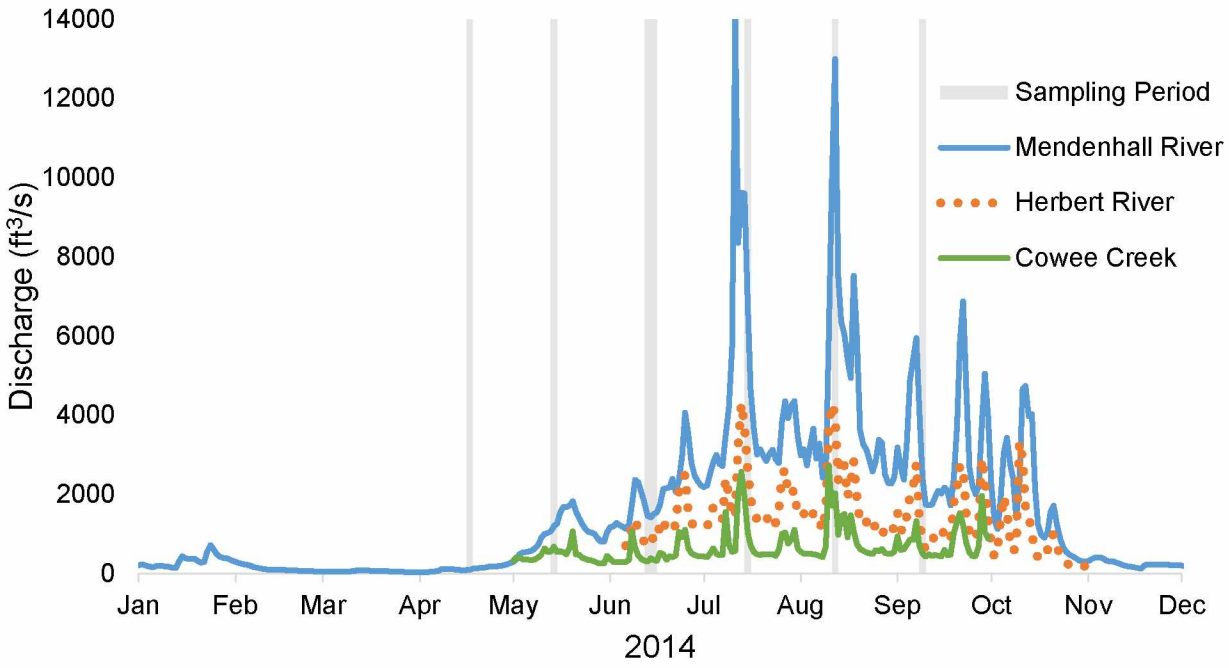


Fig. 1.2 Freshwater discharge from Cowee Creek, Herbert River, and Mendenhall River. Data are from USGS for Mendenhall River (available for January-December 2014) and from Jason Fellman, University of Alaska Southeast, for the Herbert River (available for 6/6/14-10/31/14) and Cowee Creek (available for 5/1/14-9/30/14). The Herbert River joins the Eagle River before flowing out into the Eagle River estuary. The Herbert River gage was placed upstream of the Herbert and Eagle River confluence and thus is not representative of the total discharge entering the Eagle River estuary but does capture the seasonal variability and peaks

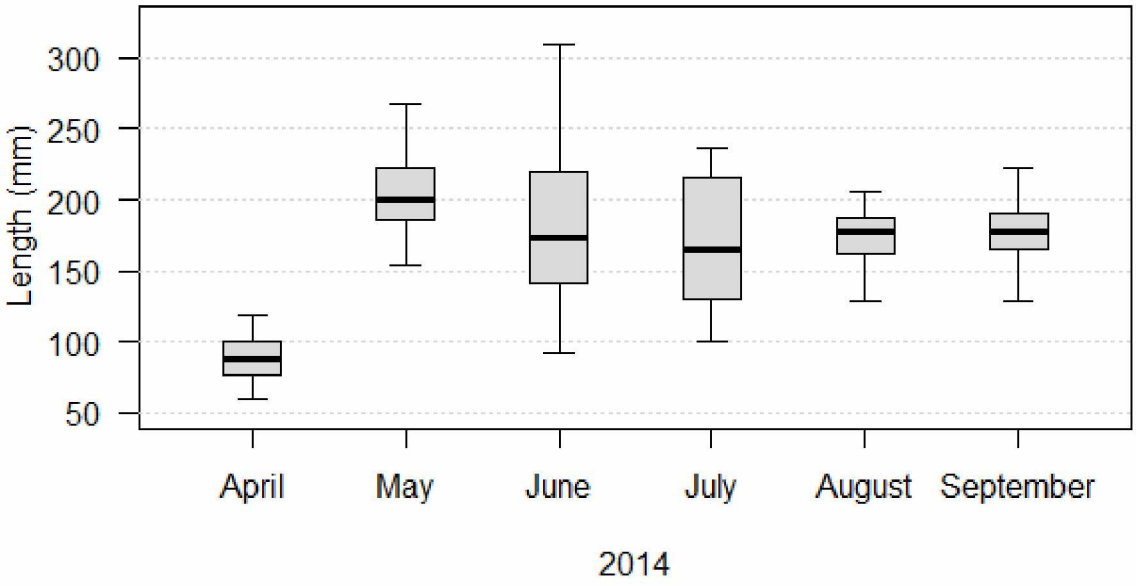


Fig. 1.3 Boxplots of the lengths (mm, total length) of staghorn sculpins retained for diet analysis during each sampling month. Boxes show the interquartile range and the whiskers represent the furthest observation within 1.5x the interquartile range of the box

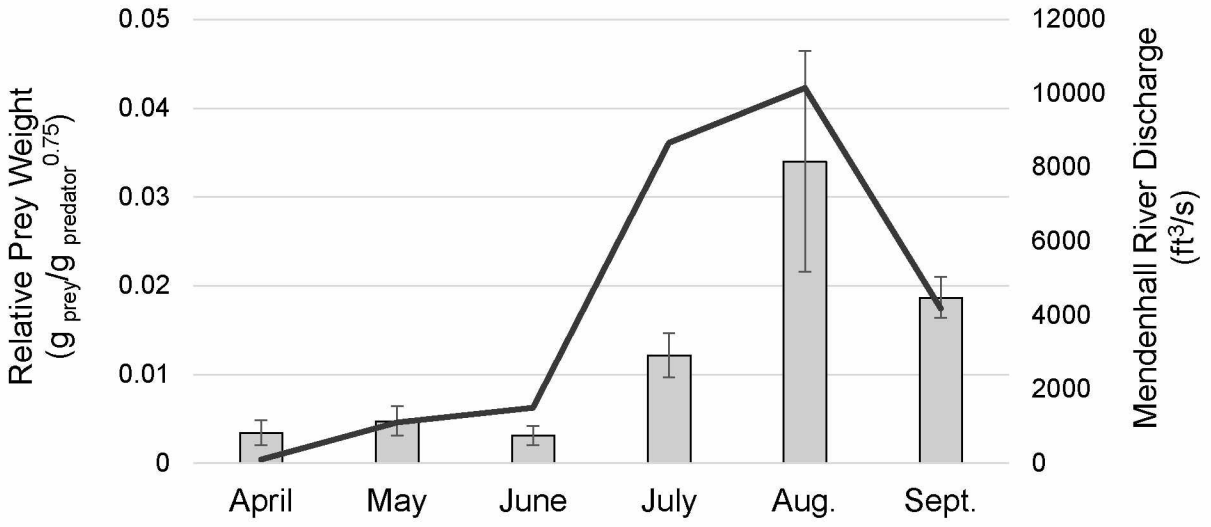


Fig. 1.4 Mean mass of mysids per stomach at the Mendenhall River estuary (bars) and Mendenhall River discharge (line) by sampling month. Whiskers show ± 1 standard error

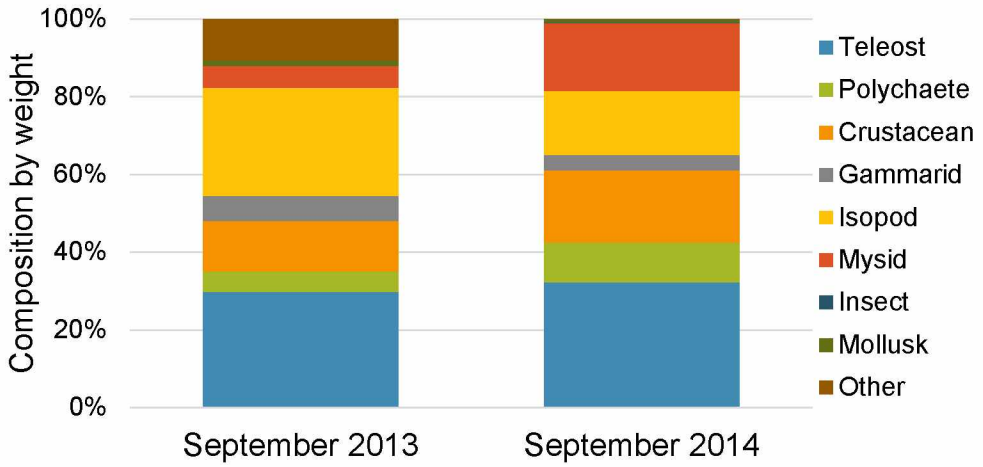


Fig. 1.5 September staghorn sculpin diet composition by weight in 2013 and 2014, combined sites

Tables

Table 1.1 Watershed characteristics of Cowee Creek, Eagle River, and Mendenhall River

Watershed Characteristics[a]	Cowee Creek	Eagle River[b]	Mendenhall River
Watershed size (km^2)	110	276	222
Glacier cover (%)	13	48-49	63
Forest cover (%)	57	23-25	8

[a] Fellman et al. 2014

[b] The Herbert River joins the Eagle River before flowing out into the Eagle River estuary, effectively doubling the watershed area draining to the estuary. The watershed area and characteristics in the table are for the combined Herbert and Eagle River watersheds, which share a similar degree of glacier and forest cover.

Table 1.2 Summary of sampling events by site (CC – Cowee Creek estuary, ER – Eagle River estuary, MR – Mendenhall River estuary), including numbers and sizes of staghorn sculpin collected and environmental conditions. Salinity, temperature, turbidity, and dissolved oxygen values are the average of three point measurements. Discharge rates for ER are from the Herbert River gage and reflect trends but not the overall volume of water entering the estuary. Discharge data are from USGS 2014 and Jason Fellman, University of Alaska Southeast

Sampling period	Site	Number of staghorn sculpin stomachs collected	Average (SD) total length of collected sculpin	Average Salinity (‰)	Average Temperature (^{0}C)	Average Turbidity (NTU)	Average Dissolved Oxygen (mg/L)	Average monthly river discharge (ft^3/s)	Discharge during sampling period (3 day average, ft^3/s)
4/18/2014	CC	6	129 (42)	31.1	4.6	2.6	13.7	-	-
5/15/2014	CC	9	190 (65)	23.5	9.5	2.3	13.1	457	598
6/12/2014	CC	8	124 (23)	18.3	12.5	4.3	10.6	494	386
7/16/2014	CC	28	138 (23)	10.9	12.8	7.0	10.4	811	1223
8/12/2014	CC	18	167 (33)	14.4	12.8	56.8	9.7	835	2154
9/10/2014	CC	25	182 (11)	16.6	11.2	8.5	9.4	781	553
4/16/2014	ER	17	90 (15)	31.2	5.5	2.5	20.2	-	-
5/13/2014	ER	28	213 (28)	22.8	10.0	2.4	14.0	-	-
6/13/2014	ER	26	205 (41)	18.9	12.7	3.0	11.7	1233	815
7/14/2014	ER	25	159 (29)	16.4	11.9	31.2	10.6	1888	3764
8/11/2014	ER	26	176 (15)	17.2	12.8	31.9	9.8	1821	3219
9/08/2014	ER	25	194 (31)	18.6	11.1	9.9	10.2	1474	2128
4/17/2014	MR	27	90 (9)	29.9	6.0	16.6	16.7	129	99
5/14/2014	MR	26	192 (15)	17.5	11.5	3.7	15.3	1027	1100
6/16/2014	MR	32	164 (28)	18.8	11.6	7.9	10.8	2006	1497
7/15/2014	MR	25	220 (9)	9.9	9.9	31.0	10.8	4399	8667
8/13/2014	MR	26	179 (10)	8.0	9.3	36.6	10.8	4325	10147
9/09/2014	MR	25	175 (16)	15.9	10.5	10.5	10.0	3243	4183

Table 1.3 Monthly diet composition of staghorn sculpin from April to September 2014, combined across sites and presented as percent frequency of occurrence (%FO) and percent by weight (%W). Prey taxa represents the lowest taxonomic level to which prey were identified. In cases of rare prey items (occurring in less than 0.25% of samples), items were grouped in an "other" category at a higher taxonomic level (e.g., "Other teleostei")

	2014														2013	
	April		May		June		July		August		September		Total		September	
Prey Taxa	%FO	%W	%FO	%W	%FO	%W	%FO	%W	%FO	%W	%FO	%W	%FO	%W	%FO	%W
Fishes																
Teleostei unidentified			6.35	11.07	36.36	18.21	30.77	11.52	22.86	0.93	14.67	1.01	19.65	9.72	4.55	3.52
Oncorhynchus					12.12	24.62							1.99	7.51		
Oncorhynchus eggs							1.28	0.20	2.86	0.16	4.00	3.88	1.49	0.63	4.55	0.27
Oncorhynchus kisutch					3.03	4.07	1.28	2.50					0.75	1.64		
Mallotus villosus					3.03	4.31					1.33	5.23	0.75	2.08		
Cottidae			1.59	0.16	10.61	1.16	5.13	0.96	4.29	1.52	1.33	0.09	3.98	0.85		
Myoxocephalus polyacanthocephalus							1.28	3.96	1.43	1.85			0.50	1.00		
Podothecus accipenserinus											4.00	1.11	0.75	0.16		
Pleuronectiformes			1.59	0.18	1.52	0.08	3.85	1.41	1.43	1.45	8.00	2.32	2.99	0.91	2.27	1.32
Pleuronectidae					3.03	0.79	3.85	0.75	14.29	1.94	17.33	11.80	6.97	2.47	2.27	0.29
Platichthys stellatus									4.29	1.89	2.67	1.74	1.24	0.62		
Lepidopsetta spp.									2.86	0.99			0.50	0.19		
Clupea pallasii			1.59	0.29	1.52	2.08			11.43	10.71			2.49	2.78	2.27	17.85
Lumpenus sagitta					10.61	1.95	1.28	0.67			1.33	2.97	2.24	1.14		
Ammodytes hexapterus			1.59	4.12	7.58	0.38	1.28	1.30	7.14	1.79			2.99	1.41		
Other teleostei			1.59	0.81	1.52	2.28					1.33	0.78	0.75	0.95	4.55	1.63

Table 1.3 continued…

	2014														2013	
	April		May		June		July		August		September		Total		September	
Prey Taxa	%FO	%W	%FO	%W	%FO	%W	%FO	%W	%FO	%W	%FO	%W	%FO	%W	%FO	%W
Invertebrates																
Polychaetes																
Polychaeta unidentified	58.00	28.47	52.38	16.53	50.00	2.31	57.69	13.12	52.86	4.75	54.67	3.31	54.23	7.56	29.55	1.25
Nereididae	4.00	3.64	20.63	22.24	6.06	0.15	5.13	5.31	15.71	25.93	8.00	1.74	9.95	10.25	18.18	3.01
Pectinaria	4.00	0.75	1.59	0.05	3.03	0.24	1.28	0.01	8.57	0.22	4.00	0.03	3.73	0.14	4.55	0.12
Echiurus					1.52	0.48	5.13	4.99	7.14	0.58	17.33	4.56	5.72	1.73	6.82	0.05
Other polychaeta											2.67	0.20	0.50	0.03		
Molluscs																
Mollusca unidentified					1.52	0.00	2.56	0.05			2.67	0.03	1.24	0.01	2.27	0.01
Bivalvia	4.00	5.14	12.70	0.45	9.09	0.17	6.41	0.06	11.43	0.10	10.67	0.11	9.20	0.24	6.82	0.41
Clinocardium	2.00	0.21	1.59	1.05			3.85	0.03	4.29	0.02	4.00	0.11	2.74	0.22		
Macoma			4.76	0.06	1.52	0.01	1.28	0.01	1.43	0.01	4.00	0.04	2.24	0.02		
Mytilus trossulus			17.46	0.22	19.70	0.18	8.97	0.08	32.86	0.39	28.00	0.22	18.66	0.21	18.18	0.48
Gastropoda			9.52	0.05	10.61	0.03	14.10	0.14	10.00	0.05	12.00	0.08	9.95	0.06	20.45	0.34
Littorina sitkana			3.17	0.05	1.52	0.00	2.56	0.03	2.86	0.02			1.74	0.02	2.27	0.01
Other mollusca			1.59	0.26	1.52	0.05			2.86	0.01	1.33	0.22	1.24	0.09	2.27	0.01
Marine Crustaceans																
Crustacea unidentified	18.00	3.75	11.11	0.85	9.09	0.47	8.97	0.28	2.86	0.11	4.00	0.06	8.46	0.42	20.45	1.47
Harpacticoida	28.00	3.37	1.59	0.03			1.28	0.01	1.43	0.01	1.33	0.01	4.48	0.05		
Cirripedia			1.59	0.05	6.06	0.06	1.28	0.02			2.67	0.03	1.99	0.03		
Malacostraca	2.00	0.21	4.76	1.58							8.00	1.10	2.49	0.45		
Peracarida					1.52	0.02			1.43	0.01			0.50	0.01		
Mysidae	36.00	10.15	53.97	3.40	42.42	1.34	75.64	14.68	90.00	19.53	78.67	16.86	64.93	9.80	75.00	4.74
Cumacea	22.00	1.17			3.03	0.01	2.56	0.02	2.86	0.01			4.23	0.02		
Isopoda unidentified			4.76	0.07	4.55	0.22	5.13	0.21	1.43	0.01	1.33	0.03	2.99	0.12	4.55	1.40
Pentidotea wosnesenskii			11.11	1.02	10.61	0.73	16.67	6.43	7.14	1.32	14.67	3.53	10.70	2.22	43.18	11.28
Gnorimosphaeroma oregonensis	2.00	2.99	34.92	4.43	42.42	5.07	46.15	14.93	48.57	4.13	49.33	12.37	39.30	7.40	72.73	10.62

Table 1.3 continued…

	2014														2013	
	April		May		June		July		August		September		Total		September	
Prey Taxa	%FO	%W	%FO	%W	%FO	%W	%FO	%W	%FO	%W	%FO	%W	%FO	%W	%FO	%W
Gammaridea	66.00	26.24	85.71	6.92	81.82	9.83	82.05	6.35	62.86	6.97	73.33	3.73	75.62	7.51	77.27	5.33
Americorophium spinicorne	6.00	0.54			22.73	0.14	2.56	0.02	7.14	0.04	5.33	0.04	7.21	0.07		
Decapoda									2.86	0.53	4.00	0.90	1.24	0.24	4.55	0.31
Other brachyurans					3.03	10.93	2.56	2.23	1.43	0.41			1.24	3.78	2.27	0.43
Caridea	2.00	4.17			3.03	0.02	16.67	0.29	12.86	0.14	30.67	4.09	11.94	0.73	18.18	2.96
Crangon	2.00	0.86	3.17	0.10	16.67	1.75	10.26	0.22	17.14	5.45	21.33	11.58	12.44	3.36	27.27	2.32
Paguroidea			7.94	3.86	9.09	2.01			4.29	0.39	1.33	0.22	3.73	1.41	4.55	0.26
Other decapoda			22.22	15.67	15.15	2.96	2.56	0.43	1.43	1.54			6.72	4.07	2.27	3.15
Insects																
Insecta unidentified			3.17	0.05	10.61	0.04	14.10	0.24	8.57	0.18	2.67	0.14	6.97	0.11	4.55	0.03
Coleoptera (adult)					4.55	0.03	1.28	0.01					1.00	0.01		
Diptera (all life stages)			19.05	0.94	15.15	0.20	17.95	0.22	17.14	0.15	1.33	0.05	12.19	0.30	4.55	0.03
Other insecta (larvae)					1.52	0.06	1.28	0.07					0.50	0.03		
Other invertebrates			1.59	0.01	1.52	0.18	1.28	0.01					0.75	0.06	11.36	8.82
Other																
Unknown	24.00	8.35	9.52	1.95	12.12	0.22	16.67	3.13	8.57	1.68	17.33	0.86	14.43	1.48	34.09	1.05
Algae or leaf litter			26.98	1.52	30.30	0.18	61.54	3.10	71.43	2.08	74.67	2.83	47.51	1.65	38.64	15.26

Table 1.4 Monthly one-way analysis of similarity (ANOSIM) R values showing temporal differences in staghorn sculpin diet composition between the Mendenhall River and Cowee Creek estuaries. We interpreted R values ≤ 0.25 to indicate weak differences, R values >0.26 and ≤ 0.50 reflected moderate differences, and R values >0.51 reflected strong differences between groups (Creque and Czesny 2012)

Month	R value
May	0.189
June	0.564
July	0.251
August	0.472
September	0.229

Table 1.5 Results of the similarity percentages (SIMPER) analyses showing the average dissimilarity between sampling periods (%) and the primary prey items that contributed to the top 50% differences

Prey taxa	CC, ER	CC, MR	ER, MR
Average Dissimilarity	78.39	76.52	71.33
Prey taxa contribution to between site differences (%)			
Mysidae	15.75	16.99	12.72
Gammaridea	11.58	11.10	13.25
Polychaeta	11.45	10.39	13.18
Gnorimosphaeroma oregonensis	5.98	10.16	9.20
Teleostei	5.92	5.50	3.82

References

Abrantes, K. G., A. Barnett, T. R. Marwick, and S. Bouillon. 2013. Importance of terrestrial subsidies for estuarine food webs in contrasting East African catchments. *Ecosphere* 4: 1–33.

Arendt, A. A., K. A. Echelmeyer, and W. D. Harrison. 2002. Rapid wastage of Alaska glaciers and their contribution to rising sea level. *Science* 297: 382–386.

Armstrong, J. L., D. A. Armstrong, and S. B. Mathews. 1995. Food habits of estuarine staghorn sculpin, *Leptocottus armatus* with focus on consumption of juvenile Dungeness crab *Cancer magister*. *Fisheries Research* 93: 456–470.

Beamish, R. J., D. J. Noakes, G. A. McFarlane, W. Pinnix, R. Sweeting, and J. King. 2000. Trends in coho marine survival in relation to the regime concept. *Fisheries Oceanography* 9: 114–119. doi:10.1046/j.1365-2419.2000.00126.x.

Beaudreau, A. H., and T. E. Essington. 2007. Spatial, temporal, and ontogenetic patterns of predation on rockfishes by lingcod. *Transactions of the American Fisheries Society* 136: 1438–1452. doi:10.1577/T06-236.1.

Butler, T. H. 1980. *Shrimps of the Pacific coast of Canada*. Ottawa: Canadian Bulletin of Fisheries and Aquatic Sciences 202.

Chipps, S. R., and J. E. Garvey. 2007. Assessment of diets and feeding patterns. In *Analysis and interpretation of freshwater fisheries data*, ed. C. Guy, 473–514. Bethesda: American Fisheries Society.

Clarke, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* 18: 117–143. doi:10.1111/j.1442-9993.1993.tb00438.x.

Clarke, K. R., and R. N. Gorley. 2015. PRIMER v7 : User manual/tutorial. Plymouth: PRIMER-E.

Clarke, K. R., R. N. Gorley, P. J. Somerfield, and R. M. Warwick. 2014. Change in marine communities: an approach to statistical analysis and interpretation. Plymouth: PRIMER-E.

Creque, S. M., and S. J. Czesny. 2012. Diet overlap of non-native alewife with native yellow perch and spottail shiner in nearshore waters of southwestern Lake Michigan, 2000-2007. *Ecology of Freshwater Fish* 21: 207–221. doi:10.1111/j.1600-0633.2011.00538.x.

Durant, J. M., D. O. Hjermann, G. Ottersen, and N. C. Stenseth. 2007. Climate and the match or mismatch between predator requirements and resource availability. *Climate Research* 33: 271–283. doi:10.3354/cr033271.

Elliott, M., and V. Quintino. 2007. The Estuarine Quality Paradox, Environmental Homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. *Marine Pollution Bulletin* 54: 640–645. doi:10.1016/j.marpolbul.2007.02.003.

Essington, T. E., J. F. Kitchell, and C. J. Walters. 2001. The von Bertalanffy growth function, bioenergetics, and the consumption rates of fish. *Canadian Journal of Fisheries and Aquatic Sciences* 58: 2129–2138. doi:10.1139/f01-151.

Fellman, J. B., S. Nagorski, S. Pyare, A. W. Vermilyea, D. Scott, and E. Hood. 2014. Stream temperature response to variable glacier coverage in coastal watersheds of Southeast Alaska. *Hydrological Processes* 28: 2062–2073. doi:10.1002/hyp.9742.

Fellman, J. B., R. G. M. Spencer, P. J. Hernes, R. T. Edwards, D. V. D'Amore, and E. Hood. 2010. The impact of glacier runoff on the biodegradability and biochemical composition of terrigenous dissolved organic matter in near-shore marine ecosystems. *Marine Chemistry* 121: 112–122. doi:10.1016/j.marchem.2010.03.009.

Herald, E. S. 1961. *Living fishes of the world.* Garden City: Doubleday and Co.

Hill, D. F., N. Bruhis, S. E. Calos, A. Arendt, and J. Beamer. 2015. Spatial and temporal variability of freshwater discharge into the Gulf of Alaska. *Journal of Geophysical Research: Oceans* 120: 634–646. doi:10.1002/2014JC010395.

Hood, E., and L. Berner. 2009. Effects of changing glacial coverage on the physical and biogeochemical properties of coastal streams in southeastern Alaska. *Journal of Geophysical Research* 114. doi:10.1029/2009jg000971.

Howe, E. R., and C. A. Simenstad. 2011. Isotopic determination of food web origins in restoring and ancient estuarine wetlands of the San Francisco Bay and Delta. *Estuaries and Coasts* 34: 597–617. doi:10.1007/s12237-011-9376-8.

Johnson, S. W., A. D. Neff, and M. R. Lindeberg. 2015. *A handy field guide to the nearshore marine fishes of Alaska.* U.S. Dep. Commer., NOAA Tech. Memo. NMFS-AFSC-293, 211 p.

Johnson, S. W., A. D. Neff, and J. F. Thedinga. 2005. *An atlas on the distribution and habitat of common fishes in shallow nearshore waters of Southeastern Alaska.* U.S. Dep. Commer., NOAA Tech. Memo. NMFS-AFSC-157, 89 p.

Johnston, N. T., and T. G. Northcote. 1989. Life-history variation in *Neomysis mercedis* Holmes (Crustacea, Mysidacea) in the Fraser River estuary, British Columbia. *Canadian Journal of Zoology* 67: 363–372.

Kathman, R. D., W. C. Austin, J. C. Saltman, and J. D. Fulton. 1986. *Identification Manual to the Mysidacea and Euphausiacea of the Northeast Pacific. Canadian Special Publication of Fisheries and Aquatic Sciences.* Vol. 93. Ottawa. doi:10.1017/CBO9781107415324.004.

Kostecki, C., F. Le Loc'h, J. M. Roussel, N. Desroy, D. Huteau, P. Riera, H. Le Bris, and O. Le Pape. 2010. Dynamics of an estuarine nursery ground: the spatio-temporal relationship between the river flow and the food web of the juvenile common sole (*Solea solea*, L.) as revealed by stable isotopes analysis. *Journal of Sea Research* 64. Elsevier B.V.: 54–60. doi:10.1016/j.seares.2009.07.006.

Kozloff, E. N. 1996. *Marine invertebrates of the Pacific Northwest.* Seattle: University of Washington Press.

Larsen, C. F., E. Burgess, A. A. Arendt, S. O'Neel, A. J. Johnson, and C. Kienholz. 2015. Surface melt dominates Alaska glacier mass balance. *Geophysical Research Letters* 42: 5902–5908. doi:10.1002/2015GL064349.

Link, J. S., and L. P. Garrison. 2002. Trophic ecology of Atlantic cod *Gadus morhua* on the northeast US continental shelf. *Marine Ecology Progress Series* 227: 109–123. doi:10.3354/meps227109.

Mace, P. M. 1983. Predator-prey funcational responses and predation by staghorn sculpin (*Leptocottus armatus*) on Chum Salmon Fry (*Onchorhynchus keta*). University of British Columbia. doi:10.1017/CBO9781107415324.004.

McAfee, S. A., J. Walsh, and T. S. Rupp. 2014. Statistically downscaled projections of snow/rain partitioning for Alaska. *Hydrological Processes* 28: 3930–3946. doi:10.1002/hyp.9934.

Mecklenburg, C. W., T. A. Mecklenburg, and L. K. Thorsteinson. 2002. *Fishes of Alaska.* Bethesda: American Fisheries Society.

Mills, K. L., T. Laidig, S. Ralston, and W. J. Sydeman. 2007. Diets of top predators indicate pelagic juvenile rockfish (*Sebastes* spp.) abundance in the California Current System. *Fisheries Oceanography* 16: 273–283. doi:10.1111/j.1365-2419.2007.00429.x.

Milner, A. M., and G. E. Petts. 1994. Glacial rivers: physical habitat and ecology. *Freshwater Biology* 32: 295–307. doi:10.1111/j.1365-2427.1994.tb01127.x.

Morris, R. W. 1960. Temperature, salinity, and southern limits of three species of Pacific Cottid fishes. *Limnology and Oceanography* 5: 175–179. doi:10.4319/lo.1960.5.2.0175.

Nakano, S., and M. Murakami. 2001. Reciprocal subsidies: Dynamic interdependence between terrestrial and aquatic food webs. *Proceedings of the National Academy of Sciences of the United States of America* 98: 166–170.

National Weather Service. 2014. Climate Report National Weather Service Juneau. Juneau: U.S. Department of Commerce, NOAA.

Neal, E. G., E. Hood, and K. Smikrud. 2010. Contribution of glacier runoff to freshwater discharge into the Gulf of Alaska. *Geophysical Research Letters* 37: L06404. doi:10.1029/2010gl042385.

O'Clair, R. M., and C. E. O'Clair. 1998. *Southeast Alaska's Rocky Shores.* Auke Bay: Plant Press.

Pasquaud, S., P. Elie, C. Jeantet, I. Billy, P. Martinez, and M. Girardin. 2008. A preliminary investigation of the fish food web in the Gironde estuary, France, using dietary and stable isotope analyses. *Estuarine, Coastal and Shelf Science* 78: 267–279. doi:10.1016/j.ecss.2007.12.014.

Roseneau, D. G., and G. V. Byrd. 1997. Using Pacific halibut to sample the availability of forage fishes to seabirds. In *Forage Fishes in Marine Ecosystems. Proceedings of the International Symposium on the Role of Forage Fishes in Marine Ecosystems. Alaska Sea Grant College Program Report No. 97-01.* University of Alaska Fairbanks.

Royer, T. C., C. E. Grosch, and L. A. Mysak. 2001. Interdecadal variability of Northeast Pacific coastal freshwater and its implications on biological productivity. *Progress in Oceanography* 49: 95–111.

Siegfried, C. A., and M. E. Kopache. 1980. Feeding of *Neomysis mercedis* (Holmes). *Biological Bulletin* 159: 193–205.

Sklar, F. H., and J. A. Browder. 1998. Coastal environmental impacts brought about by alterations to freshwater flow in the Gulf of Mexico. *Environmental Management* 22: 547–562. doi:10.1007/s002679900127.

Stabeno, P. J., N. A. Bond, A. J. Hermann, N. B. Kachel, C. W. Mordy, and J. E. Overland. 2004. Meteorology and oceanography of the Northern Gulf of Alaska. *Continental Shelf Research* 24: 859–897. doi:10.1016/j.csr.2004.02.007.

Tasto, R. N. 1975. *Aspects of the biology of the Pacific staghorn sculpin, Leptocottus armatus Girard, in Anaheim Bay. The Marine Resources of Anaheim Bay.*

U.S. Geological Survey (USGS). 2014. National water information system: Web interface: USGS 15052500 Mendenhall R NR Auke Bay AK.

Vinagre, C., J. Salgado, H. N. Cabral, and M. J. Costa. 2011. Food web structure and habitat connectivity in fish estuarine nurseries-impact of river flow. *Estuaries and Coasts* 34: 663–674. doi:10.1007/s12237-010-9315-0.

West, J. M., G. D. Williams, S. P. Madon, and J. B. Zedler. 2003. Integrating spatial and temporal variability into the analysis of fish food web. *Environmental Biology of Fishes* 67: 297–309.

Willson, M. F., and A. P. Baldwin. 2003. *Invertebrate surveys on the Mendenhall Wetlands.* Juneau, Report to U.S. Fish and Wildlife Service.

Chapter 2: Using stable isotopes to assess the contribution of terrestrial and riverine organic matter to estuarine food webs in a glacial fjord ecosystem[1]

Abstract

Terrestrial and marine ecosystems in Southeast Alaska (SEAK) are linked by the flow of freshwater from precipitation and glacial runoff, which transports nutrients and organic matter (OM) of terrestrial and riverine origin downstream to estuaries. Allochthonous OM may be an important subsidy to estuarine consumers, particularly in SEAK, where the flux of terrestrial OM to the nearshore marine environment is high. However, understanding the contribution of allochthonous OM to estuary food webs requires a place-based approach, since the use of terrestrial-riverine OM by marine species has been shown to vary across systems. Here, we examined the relative importance of terrestrial-riverine and marine OM to fish and invertebrates collected from three glacially-influenced estuaries in SEAK. Multiple stable isotope tracers ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) were used to examine dietary sources of consumers between spring and early fall. We found limited use of terrestrial-riverine OM by estuarine fishes, however, marine species (staghorn sculpin, *Leptocottus armatus*, and starry flounder, *Platichthys stellatus*) relied less on allochthonous OM than anadromous species (Dolly Varden, *Salvelinus malma*, and coho salmon, *Oncorhynchus kisutch*). Invertebrates showed greater use of terrestrial-riverine OM as well as greater temporal variation in OM use than fish. Invertebrates sampled from the site with the lowest glacial coverage incorporated more terrestrial-riverine OM than organisms collected from sites with higher glacial coverage. The linkages between these habitats and the relative importance of terrestrial-riverine OM to estuarine consumers is especially important to understand given the increases in temperature and precipitation that are predicted for SEAK in

[1] Whitney, E. J., A. H. Beaudreau, E. R. Howe. 2016. Prepared for submission to Estuaries and Coasts.

the coming decades, which will likely alter the flux of OM between the terrestrial, riverine, and marine environments.

Introduction

Allochthonous subsidies of nutrients and organic matter (OM) delivered from one habitat to another can greatly affect food web structure and function in the recipient habitat (Polis et al. 1997). The importance of terrestrial-riverine OM to nearshore marine species has been documented across a range of coastal ecosystems (Nixon et al. 1986; Bouillon et al. 2000; Connolly et al. 2009) and the magnitude of the subsidy can vary as a function of river discharge. For instance, Hoffman et al. (2007) found that young-of-the-year shad in estuaries shifted from a reliance on autochthonous resources during years of low river discharge to primarily terrestrially-derived OM during periods of high river flow in Mattaponi River, Virginia. Across the Atlantic, in the northwest Mediterranean, variation in sole fishery yields was linked to variable terrestrial particulate organic matter (POM) contributions to benthic food webs arising from fluctuating river flow (Darnaude et al. 2004b). The literature is equally populated, however, with studies indicating that terrestrial OM does not contribute substantially to estuarine production and varies in its importance as a resource among species and ecosystems (Darnaude et al. 2004a; Martineau et al. 2004; Franca et al. 2011). For example, estuarine consumers in Plum Island Sound, Massachusetts, did not rely heavily on terrestrially-derived riverine OM, even in areas where it was most available (Deegan and Garritt 1997). Abrantes et al. (2013) found the importance of terrestrial OM depends on watershed characteristics, with greater use in C4 plant dominated landscapes compared to C3 plants. In addition, our understanding of OM use is likely affected by the species examined and the feeding behavior of the selected species. Collectively, these studies

reveal that the relative importance of terrestrial and freshwater derived OM varies among and within systems. Because estuarine consumers may show variable responses to allochthonous inputs and site characteristics, understanding how changes in terrestrial-riverine OM will affect estuarine food webs requires a place-based approach (Fry 2002; Franca et al. 2011).

The substantial flow of freshwater into the Gulf of Alaska (GOA, 849 km^3yr^{-1}; Hill et al. 2015) transports bioavailable OM to marine habitats and affects the dynamics of OM use in this region. Each year 0.13±0.01 Tg of dissolved organic carbon (DOC), a component of total OM, is delivered to the GOA in glacial meltwater (Hood et al. 2009). Along the eastern GOA, the riparian forests and vegetated mountain slopes of the Pacific coastal temperate rainforest (PCTR) could contribute substantial OM to streams that feed local estuaries (Fellman et al. 2015). Shallow soils and steep valley walls along the PCTR further facilitate the downstream transport of OM (Wipfli 2005). Given the permeable boundaries between the terrestrial and marine environments (Polis et al. 1997) and the landscape features that link these environments, terrestrial OM may provide an important subsidy to estuarine consumers along the PCTR. This hypothesis, however, has not been widely addressed in glacially-influenced estuaries (but see Arimitsu 2016).

This study evaluated the contribution of terrestrial-riverine OM to consumers in estuaries of Southeast Alaska (SEAK) in the PCTR, which represent a class of high latitude, glacially-influenced estuaries that are understudied compared to those in lower latitudes that are significantly influenced by human activities. Not only are SEAK's estuaries largely undeveloped, but they are also subject to rapid change due to melting and retreating glaciers (Larsen et al. 2007). In the 12-volume Treatise on Estuarine and Coastal Science (Wolanski and McLusky 2011), one of the most up-to-date references on estuarine and coastal science and

management, an entire volume is devoted to research on systems affected by development and other human activities (Volume 8; Wolanski and McLusky 2011) but there are only limited references to coastal systems currently affected by glacial ice (but see Forbes 2011). Further, in SEAK, the proximity of glacial headwaters to the coast results in short transit times of freshwater and OM to estuaries. The average length of streams with glacial headwaters in SEAK is just 10 km, two orders of magnitude shorter than the average for the Western United States (O'Neel et al. 2015). This geographical compression increases the velocity of river effluent and alters the way in which sediment and other particulates are delivered to estuaries (Syvitski et al. 2005). These aspects set SEAK estuaries apart from other longer, non-glacially-influenced estuaries.

The study objectives were to (1) evaluate the relative contribution of terrestrial-riverine and marine OM sources to the diets of estuary fishes and invertebrates and (2) examine spatial and temporal variation in terrestrial-riverine and marine OM contributions to consumers across three SEAK estuary sites and six sampling months. To address these objectives, we used a multiple stable isotope tracer approach to determine the relative contribution of each source to the diets of consumers (Hobson 1999; Darnaude et al. 2004a; Pasquaud et al. 2007). Sources can be partitioned using multiple stable isotopes (i.e., ^{13}C, ^{15}N, ^{34}S) based on the stable isotope signature of the consumer relative to the signature of the source material (DeNiro and Epstein 1978; Peterson et al. 1985). Carbon and sulfur isotopes both provide information on the source of OM assimilated by the organism, while nitrogen indicates source as well as the trophic level of the organism (DeNiro and Epstein 1987, Peterson and Fry 1987). Sulfur isotopes are less commonly used but can help distinguish between coastal sources when carbon signatures are similar (Connolly et al. 2004). Using these three stable isotopes together strengthens the isotopic

tracer approach and provides more power to address food web structure and OM sources (Peterson et al. 1985; Connolly et al. 2004).

We hypothesized that the importance of allochthonous terrestrial-riverine OM relative to autochthonous marine OM sources would vary across estuarine species. Invertebrate species, particularly benthic feeders, may show greater use of terrestrial-riverine OM than higher trophic level fish based on research indicating that terrestrial OM enters marine food webs through benthic pathways (Darnaude et al. 2004b). Among estuarine fishes, diet data (Whitney 2016, Whitney unpublished) informed our hypothesis that anadromous fishes would incorporate both terrestrial-riverine and marine sources, whereas marine fishes would be less reliant on terrestrial-riverine sources. We expected to see differences in where and when terrestrial-riverine OM was important. River flow into estuaries varies seasonally and is impacted by watershed characteristics, such as the extent of glacial and forest coverage (Hood and Berner 2009; Neal et al. 2010; Fellman et al. 2014). As such, we expected that the contribution of terrestrial-riverine OM would be highest in the summer during the peak discharge period for glacier-fed watersheds and highest in late spring or early fall during peak flows in predominately rain-fed watersheds. However, we expected these responses to freshwater discharge to be delayed several weeks due to tissue-turnover (Church et al. 2009; Heady and Moore 2013).

Methods

Study sites

This study was conducted at three estuary sites near Juneau, AK: Cowee Creek estuary (CC; 58.682 °N, -134.951 °W), Eagle River estuary (ER; 58.539 °N, -134.85 °W), and Mendenhall River estuary (MR; 58.328 °N, -134.607 °W). These sites are located along Lynn

Canal, a glacial fjord in SEAK in the PCTR, which receives a yearly average of 1400 mm of precipitation at sea level (Fellman et al. 2015). Watershed composition ranges from 63% glaciated and 8% forested (MR) to 13% glaciated and 57% forested, (CC; Table 2.1, Fellman et al. 2014). The confluence of the Herbert River and Eagle River flows into the ER estuary site. Together the Herbert and Eagle River watersheds are intermediate to MR and CC in glacial and forest coverage (Table 2.1). CC also has more extensive low gradient floodplain channels compared to either MR or ER (CBJ 2016). The delivery of freshwater helps shape the physical characteristics of these estuaries. Peak discharge during sampling months occurred in mid-summer for each of the rivers, owing to higher contributions of glacial meltwater with warmer air temperatures. Additionally, the summer (June, July, and August) of 2014 was particularly wet in Juneau, receiving 616 mm out of the 1745 mm that fell in 2014 (National Weather Service 2014).

Field protocol

Estuarine fishes were collected monthly from April to September 2014 by beach seine. Sampling occurred in the estuary adjacent to the river mouth along shallow, sloping, intertidal areas with fine sand and mud sediments and interspersed cobble. During low tide windows (< 0 m below MLLW), a seine net (15 m x 2.4 m net with 6.3 mm square mesh) was walked parallel to the shoreline for 4-6 minutes before being closed and pulled onto the beach. Based on the catch composition during pilot sampling in 2013, four abundant estuarine fish species representing different feeding guilds and life history types were selected for stable isotope analysis: Pacific staghorn sculpin (*Leptocottus armatus*), starry flounder (*Platichthys stellatus*), Dolly Varden (*Salvelinus malma*), and juvenile coho salmon (*Oncorhynchus kisutch*). These taxa

represent anadromous (coho salmon and Dolly Varden) and marine species (staghorn sculpin and starry flounder), which exhibit opportunistic and generalist feeding behaviors. Muscle tissue from five starry flounder (mean length: 210 mm TL ±43, n=90) and five staghorn sculpins (mean length: 162 mm TL ±39.8, n=90) were sampled monthly at each site. Dolly Varden (mean length: 183 mm FL ±52, n=47) and juvenile coho salmon (93.3 mm FL ±11.84, n=30) were more variably present in the estuaries and the smaller sample size reflects their absence from individual sites or months (Table 2.2).

Common prey of the four fishes were collected to examine the relative importance of allochthonous and autochthonous OM sources across multiple trophic levels. Primary consumers collected included blue mussels (*Mytilus trossulus*) and isopods (*Gnorimosphaeroma oregonensis*). Omnivores included gammarids (suborder Gammaridea) and invertebrate predators included *Crangon* shrimp, hairy hermit crab (*Pagurus hirsutiusculus*), and nereid worms (family Nereididae). Intertidal invertebrates were collected by hand and dip net during low tides following beach seining in June and August.

Primary producers representing marine OM sources to estuaries were collected from each of the sites in June, July, and August. Samples of the attached marine macroalgae, *Fucus distichus evanescens*, were collected by hand from intertidal rocks. This species was selected because it is a common and abundant intertidal species (Lindeberg and Lindstrom 2010) and the dominant marine vegetation observed at our sites; although eelgrass (*Zostera marina*) is present in the region, it was absent from our sites.

Suspended terrestrial-riverine POM ≥ 250 μm, including leaf litter, was collected approximately 1-2 km upstream of the river mouth using a 250-μm mesh drift net. The net was anchored underwater close to the riverbed for 0.25-24 hours, depending on the amount of

material collected in the net (Wipfli and Gregovich 2002). Samples were collected from CC in May, July, and September and from MR in September only. Samples collected upstream of the Herbert River/Eagle River confluence were primarily composed of sediment and low OM concentrations precluded stable isotope analyses. Instead, literature stable isotope values were used for POM and leaf litter collected from lower Herbert River in 2012 (Fellman et al. 2015; data provided by Jason Fellman, University of Alaska Southeast, March 2016). Collectively, these samples represent terrestrial and riverine OM.

Stable isotope measurements

Fish were rinsed in deionized water and muscle tissue was excised from above the lateral line just posterior to the head. Muscle tissue was removed from a consistent region of the body across individuals to reduce bias associated with biochemical heterogeneity, such as in lipid composition of different tissues (Michener and Kaufman 2007). For juvenile coho salmon, muscle tissue above the lateral line along the length of the body was used. Tissue samples were then frozen until they were prepared for stable isotope analysis. Lipids were not extracted from tissue samples because lipid extraction may alter $\delta^{15}N$ in unpredictable ways (Søreide et al. 2006; Ingram and Matthews 2007). We used muscle tissue from invertebrates, removing internal organs (Hill and McQuaid 2011) and hard structures to minimize carbonate contamination in samples (Goering et al. 1990). Multiple small invertebrates were combined to create a single sample as needed to attain the minimum weight for analysis. POM samples were rinsed and sorted to remove insects. *Fucus* sp. samples were rinsed in deionized water and epiphytes removed prior to drying. All samples were placed in a drying oven at 60 ^{0}C for 48-72 hours and dried to a stable weight.

In preparation for stable isotope analysis, each sample was ground into a homogeneous powder using a mortar and pestle. Samples were weighed to the nearest 0.01 mg using a micro-analytical balance and packaged in tin capsules. Isotopic ratios of samples were analyzed for carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$), using an isotope radio mass spectrometer at the Colorado Plateau Stable Isotope Laboratory (http://www.isotope.nau.edu/index.html).

Isotope ratios are reported using the δ notation, which indicates the ratio of the heavy isotope to the light isotope relative to a standard. It is defined by the formula:

$$\delta X(‰) = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \cdot 1000 \qquad \text{Equation 2.1}$$

where X is the heavy isotope and R is the ratio of $^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{34}S/^{32}S$. The Peedee Belemite fossil formation was used as the standard for ^{13}C (Spiker and Schemel 1979). The standard for ^{15}N was atmospheric nitrogen and Canyon Diablo troilites were used for ^{34}S (Peterson and Howarth 1987). To check for consistency in sample preparation, we analyzed duplicate samples prepared from the same individual for 10% of our samples (R^2>0.97 for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$).

Statistical analyses

Differences in stable isotope signatures across species, sites, and sampling months reflect differential feeding behaviors among organisms in the proceeding weeks to months (Church et al. 2009; Heady and Moore 2013). These differences can be interpreted relative to basal sources. Marine sources tend to be enriched in ^{13}C, ^{34}S, and ^{15}N compared to terrestrial or freshwater sources (Hobson 1990; Connolly et al. 2004; Montoya 2007), thereby creating a terrestrial to marine continuum in stable isotope signatures. Species in different places along this continuum rely on contrasting proportions of the two end member sources.

We used multivariate analyses to compare consumer isotope signatures ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) by site, month, and species. Principal component analysis (PCA) plots were used to explore species and site relationships. Analysis of similarity (ANOSIM) tests were used to test for differences in stable isotope signatures of consumers among sites and months. We performed a three-way ANOSIM on a similarity matrix constructed using a Euclidean distance measure (Clarke et al. 2014) to examine overall species, site, and month differences for fish and inverts separately. This was done to broadly address to what species, where, and when terrestrial-riverine OM may be important. Differences across sites and months were examined individually by species using a two-way ANOSIM with site and month as factors. Pairwise tests within ANOSIM indicate the degree of separation among factor levels. ANOSIM produces an R statistic, which indicates the degree of separation between groups; R values range from 0 to +1. The greater the distance from zero, the more different groups are from one another. We described weak differences as R values ≤ 0.25, moderate differences were indicated by R values >0.26 and ≤ 0.50, and strong differences between groups were indicated by R values >0.51 (Creque and Czesny 2012). Multivariate analyses were conducted in PRIMER V7.0 (Plymouth Marine Laboratories, UK, Clarke and Gorley 2015).

Relative contributions of terrestrial-riverine and marine OM sources were probabilistically determined using MixSIAR, a Bayesian mixing model designed to account for variability and uncertainly in values (Moore and Semmens 2008; Stock and Semmens 2015). Carbon and sulfur stable isotopes are both used to distinguish between OM sources, sulfur being particularly useful when primary source carbon signatures are indistinct from one another (Connolly et al. 2004). In our case, primary sources could be separated based on carbon signatures and, thus, only $\delta^{13}C$ and $\delta^{15}N$ values were used as inputs into the mixing models. We

ran separate models for each species using iterative Markov chain Monte Carlo sampling with site as a fixed effect and month as a nested random effect (Semmens et al. 2009) to produce a proportional contribution estimate for each species and site. Due to small sample sizes for primary sources, we used mean isotopic signatures across sites and months. Starting trophic enrichment (fractionation) factors (TEF, mean ±SD) were assigned based on McCutchan et al. (2003) for non-lipid extracted muscle tissue (1.1 ±1.26 for $\delta^{13}C$ and 2.8 ±1.39 for $\delta^{15}N$). The TEF were then adjusted for consumers based on trophic position. To capture two trophic level shifts, we followed the methods of Vander Zander and Rasmussen (2001) to sum the TEF and variances. The final TEF values used for fish were (1.5 ±1.74 for $\delta^{13}C$ and 5.1 ±2.12 for $\delta^{15}N$) and the values used for individual invertebrate species are shown in Table 2.5. We used uninformative priors due to a lack of existing data on source contributions to consumer diets and a multiplicative error structure (residual and process error modeled separately) to add biological realism and flexibility to the likelihood error structure (Brice Semmens, Scripps Institute of Oceanography, personal communication 4/11/16). Gelman-Rubin diagnostics were used to confirm model convergence (Gelman and Rubin 1992).

Prior to running mixing models for each species, we evaluated the isospace geometry to determine whether consumer data were within the bounds of the source polygon created by the isotopic signatures of marine and terrestrial/riverine sources (adjusted for TEF values and source variation; Fig. 2.1). Consumer data well outside the polygon violates mixing model assumptions and may be an indication of a missing source (Stock and Semmens 2015). The strong negative carbon values of coho salmon fell outside of the source polygon (Fig. 2.1d) and, in order to avoid violating assumptions, a mixing model was not run for this species. Similarly, there was one Dolly Varden outlier whose carbon value ($\delta^{13}C$: -39.17‰; Fig. 2.1c) was strongly depleted

relative to other Dolly Varden samples and source materials and for this reason was excluded from the mixing model.

Results

Isotopic separation of allochthonous and autochthonous primary sources

The isotope values for the marine macroalgae, *Fucus* sp., showed a distinct marine signature (Table 2.3). The $\delta^{13}C$ values for *Fucus* sp. (-16.9 ± 1.07‰) were similar but slightly enriched compared to values for phytoplankton (-18.1 ± 0.2‰) collected during June in 1985-1986, in Auke Bay, in close proximity to MR (Goering et al. 1990). Further, the $\delta^{34}S$ in *Fucus* sp. (-22.73 ± 0.16‰) aligns with values for seawater sulfate; generally $\delta^{34}S$ is between 20-22‰, with an ocean average of 21.75 ± 0.02‰ (Szabo et al. 1950). In contrast to *Fucus* sp., the POM samples reflect a mixture of terrestrial and riverine sources (Finlay 2001). The C:N ratios of the POM (22.64 -34.05) are consistent with the range of values reported for terrestrial C3 plants and freshwater macrophytes (Finlay and Kendall 2007). The POM samples, though variable, were substantially depleted in $\delta^{13}C$ (-28.03 ±1.01‰) and $\delta^{15}N$ (0.47 ±1.43‰) relative to *Fucus* sp. (Table 2.3).

Differences in OM sources among consumers

We analyzed $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in muscle tissue samples of 257 fish and 99 invertebrates (Table 2.4). The intertidal invertebrates sampled were enriched in $\delta^{13}C$ relative to the POM samples and generally grouped closely to *Fucus* sp. values (Table 2.4, Fig. 2.2). Invertebrate species were strongly different from one another (three-way ANOSIM; R=0.858, p=0.0001). The stable isotope signatures of invertebrates showed lower variation across samples

of the same species compared to fish (Fig. 2.2), likely due to combining multiple individuals in a single sample. Marine fishes, staghorn sculpin ($\delta^{13}C$: -16.01 ±1.46‰) and starry flounder ($\delta^{13}C$: -15.64 ±1.27‰), were enriched in $\delta^{13}C$ relative to anadromous Dolly Varden ($\delta^{13}C$: -20.37 ±3.28‰) and juvenile coho salmon ($\delta^{13}C$: -26.5 ±5.63‰); all four fish species were enriched in $\delta^{13}C$ relative to POM (-28.03 ±1.01‰; Fig. 2.1). Coho salmon carbon signatures were highly variable; however, fish length poorly explained this variation (R^2=0.05, p=0.21). Sulfur signatures of the fish species followed a similar pattern as carbon (Table 2.4, Fig. 2.2). The smallest fish we analyzed, coho salmon, had the lowest $\delta^{15}N$ value ($\delta^{15}N$: 10.07 ±1.85‰) and staghorn sculpin had the highest ($\delta^{15}N$: 13.07 ±0.69‰). Dolly Varden ($\delta^{15}N$: 12.52 ±1.17‰) and starry flounder ($\delta^{15}N$: 10.07 ±1.85‰) were intermediate in nitrogen signatures. Fish and invertebrate species were highly enriched by 6.98-13‰ relative to the $\delta^{15}N$ of POM (0.47 ±1.43‰).

There were strong differences in stable isotope signatures among fish species (three-way ANOSIM; R=0.583, p=0.0001), indicating variation in their use of allochthonous and autochthonous resources. The greatest differences among the four fishes were between starry flounder and juvenile coho salmon (three-way ANOSIM pairwise; R=0.838, p=0.0001). The PCA plot, based on stable isotope signatures of fish, showed a separation of obligate marine species (staghorn sculpin and starry flounder) from anadromous species (Dolly Varden and coho salmon; Appendix 2.A). This pattern is supported by ANOSIM pairwise tests, which indicated greater differences between marine and anadromous groups (R=0.75 to 0.838, p=0.0001) than within each group (R=0.401 to 0.406, p=0.0001).

Based on mixing models, diets of primary consumers (blue mussels and isopods) were variable and composed of 16-60% terrestrial-riverine OM (range of median values across sites,

Table 2.5), with the highest contributions in isopods at ER. Omnivore invertebrate (gammarid) diets were composed of 3-20% terrestrial-riverine OM (range of median values across sites, Table 2.5). Invertebrate predators (*Crangon* shrimp, hairy hermit crab, and nereids) showed variability in source contributions, with 3-40% of their diet composed of terrestrial-riverine OM (range of median values across sites, Table 2.5). The 95% credible intervals (CI) were large for both invertebrate (Table 2.5) and marine fish mixing models (Fig. 2.3). The mixing model suggested that only 7-16% of starry flounder diet was composed of terrestrial-riverine OM (range of median values across sites, Fig. 2.3). Staghorn sculpin diets were estimated to be composed of 16-22% terrestrial-riverine OM (range of median values across sites, Fig. 2.3). Dolly Varden diets were estimated to be composed of 29-41% terrestrial-riverine OM sources (range of median values across sites, Fig. 2.3), with the highest contributions at ER.

Seasonal and spatial variation in OM sources

When we examined all fish species together for evidence of seasonal variation relative importance of terrestrial-riverine OM, we found a weak difference among sampling months (three-way ANOSIM; R=0.141, p=0.0001). Individually, starry flounder showed weak differences among months (two-way ANOSIM; R=0.203, p=0.0001). There was monthly variation in $\delta^{13}C$ and $\delta^{34}S$ signatures of starry flounder samples of up to 3.2‰ and 4‰ respectively, but with no clear seasonal trend. Staghorn sculpin showed weaker differences across months (two-way ANOSIM; R=0.094, p=0.005) without a discernable temporal trend. Dolly Varden (two-way ANOSIM; R=0.085, p=0.079) and coho salmon (two-way ANOSIM; R=0.172, p=0.074) showed no significant temporal variation. In contrast to the limited temporal variation observed in fish, the difference among sampling months for invertebrates was twice

that of fish (three-way ANOSIM; R=0.352, p=0.0001). These differences were driven by isopods and nereids that were depleted in $\delta^{13}C$ (0.92-1.34‰ depletion) and $\delta^{15}N$ (0.64-0.70‰ depletion) in June relative to their counterparts sampled in August.

There were moderate differences among sites in fish consumers' use of OM (three-way ANOSIM pairwise; R=0.375, p=0.0001). We found the strongest differences between fish from MR and CC (three-way ANOSIM pairwise; R=0.551, p=0.0001). The site-specific patterns of OM use differed between marine and anadromous species. For both staghorn sculpin and starry flounder, ER fish more closely resembled fish from CC than fish from MR. For Dolly Varden and coho salmon, ER fish more closely resembled fish from MR than fish from CC. Marine fish from MR were enriched in $\delta^{13}C$ and $\delta^{15}N$ relative to those from CC (Table 2.4). Conversely, anadromous fishes from MR were depleted in $\delta^{13}C$ and $\delta^{15}N$ relative to those from CC (Table 2.4).

Invertebrate stable isotope signatures showed strong site differences (three-way ANOSIM, R=0.71, p=0.0001), much more pronounced than differences observed among fish across sites. As with fish, the samples from CC and MR were the most distinct (three-way ANOSIM; CC/MR pairwise test; R=0.866, p=0.0001). Overall, invertebrates were generally enriched in $\delta^{13}C$ and $\delta^{15}N$ at MR compared to CC.

Discussion

Use of allochthonous OM by estuarine consumers

This study demonstrated that both terrestrial-riverine and marine OM sources are utilized by estuarine consumers. The majority of marine fish diets derive from autochthonous sources with allochthonous sources playing a limited, yet variable role. Among fish, anadromous Dolly

Varden demonstrated the highest use of allochthonous OM, with 29-41% of the diet coming from terrestrial-riverine sources compared to 16-22% for staghorn sculpin and 7-16% for starry flounder. Marine invertebrate use of allochthonous OM was variable among species (Table 2.5) but aside from mussels and gammarids, invertebrates showed greater use of terrestrial-riverine OM than marine fishes. Based on the apparent volume of OM entering these watersheds, we expected estuarine fish use terrestrial-riverine OM to be similar to that of invertebrates for whom a sizable portion of their diets was from allochthonous sources, such as *Crangon* shrimp (37-40% terrestrial-riverine OM) and nereids (39-44% terrestrial-riverine OM), but this was not the case. This finding is consistent with studies demonstrating limited use of terrestrial OM by estuarine fish (Deegan and Garritt 1997; Martineau et al. 2004; Connolly et al. 2009).

The differences in OM use among marine and anadromous fishes as well as invertebrates indicate a multispecies approach is needed to understand OM use in estuaries. Stable isotope signatures were strongly different between marine and anadromous fishes. Anadromous fishes showed greater individual variation compared to marine fishes, reflecting differences in foraging, with some individuals incorporating a higher percentage of terrestrial-riverine OM than others do. Anadromous fish move between habitats based on life stage (e.g., out-migrating coho salmon) or to take advantage of feeding opportunities, such as Dolly Varden making feeding forays into freshwater in pursuit of salmon eggs (Jaecks and Quinn 2014). This movement likely contributes to the observed variability and at times high allochthonous OM use. In contrast, starry flounder and staghorn sculpin show less variability in stable isotope signatures and lower incorporation of terrestrial-riverine derived OM (Fig. 2.1), suggesting similar feeding behaviors among individuals with a focus on marine derived prey. The separation in allochthonous OM

usage between marine and anadromous species supports our hypothesis that there are variable patterns of terrestrial-riverine and marine OM use among fish residing in estuaries.

For juvenile coho salmon that likely recently left the freshwater, the timing of their outmigration and estuarine residence time are confounding factors in interpreting their variable stable isotope signatures and OM use. Additionally, the presence of hatchery released juvenile coho salmon near ER may contribute to variability in stable isotope signatures. Not all hatchery coho salmon are marked, making it challenging to distinguish between hatchery and wild fish in the estuary. Moreover, strong negative carbon values of coho salmon were well below our measured values of riverine POM, likely reflecting feeding on a source not sampled in this project. Literature values suggest freshwater periphyton or phytoplankton as $\delta^{13}C$ depleted sources to coho salmon (Marty and Planas 2008). Additionally, the $\delta^{15}N$ values for coho and other species were slightly enriched relative to the marine source even after adjustment for trophic enrichment (Fig. 2.1) suggesting an additional marine source with a higher $\delta^{15}N$ value than the source included in our two end member model.

Marine invertebrate use of allochthonous OM was also highly variable. Some species (gammarids and blue mussels) demonstrated limited use of terrestrial-riverine OM, while other species (isopods, *Crangon* shrimp, hermit crab, and nereids) incorporated moderate amounts of allochthonous OM into their diets (22-60%). The increased use of terrestrial-riverine OM by marine invertebrates compared to marine fish suggests that this material is incorporated to a greater extent by benthic and epibenthic species at the base of food webs. This finding is supported by research indicating that dissolved OM is readily incorporated by primary consumers (Franca et al. 2011; Bell et al. 2016). Epibenthic and benthic prey make up a large portion of the diets of staghorn sculpin and starry flounder from these sites; however, they also

consume a variety of juvenile fishes (Whitney 2016), which may dilute the allochthonous OM signature. We recognize that the life history, body size, and species examined may alter our understanding of the role of terrestrial-riverine OM sources in estuaries. While the trophic ecology of estuarine fish and invertebrates does link the terrestrial and riverine environments with the marine environment, the connection may not be as obvious or as important as we hypothesized for glacially fed systems in SEAK.

Seasonal patterns in allochthonous OM use

Given the fluctuation in river discharge at each of our three sites over the course of the sampling period (Whitney 2016), we hypothesized that there would be temporal shifts observed in the stable isotope data. Invertebrates showed greater use of terrestrial-riverine OM in June (below peak discharge) compared to August (near peak discharge; Whitney 2016) based on stable isotope signatures. This result contrasted with our hypothesis that allochthonous OM use would be highest following peak flows but is supported by research indicating that benthic algae in glacial rivers and macroinvertebrate productivity has been found to be highest in spring and fall, outside the window of peak glacial runoff (Milner and Petts 1994; Brittain and Milner 2001). This suggests that riverine OM may be more available to estuarine consumers outside of summer months and explains $\delta^{13}C$ depletion in invertebrates from June compared to August.

Temporal shifts in fish OM use were less pronounced and did not show increased allochthonous OM use following peak flow periods. While there were slight but significant shifts in the stable isotope signatures of staghorn sculpin and starry flounder over the sampling period, no directional trend was detected. No significant shifts were identified for Dolly Varden or coho salmon. The lack of trends may be a result of the high in-situ productivity of the estuary. Given

the abundance of food resources available, allochthonous OM appears to be incorporated in fish diets at a consistent level rather than following predicted seasonal trends in availability. Complete tissue turnover in fish muscle can take weeks to months (e.g., 94 days for $\delta^{15}N$ for sea-run rainbow trout, *Oncorhynchus mykiss*; Church et al. 2009), however, shifts in isotopic signatures due to dietary changes can be detected sooner (Heady and Moore 2013). Given the extended period of higher freshwater discharge in the study estuaries between July and September (Whitney 2016) relative to earlier sampling months, we expected there would be sufficient time under the higher flow regime for any associated diet shifts to be detectable in muscle tissue; however, no defined seasonal pattern was detected. For coho salmon, although the combined carbon, nitrogen, and sulfur signatures did not show a significant temporal trend, $\delta^{13}C$ isotope values indicate an increase in use of marine OM as the summer progressed (May [mean ±SD] -28.88 ±6.41; June -26.02 ±4.15; July -19.42 ±1.46). This increase reflects dietary shifts that occur with smolting and increased estuarine residence time and, in turn, higher predation on marine prey.

Site characteristics contributing to allochthonous OM use

Beyond the seasonal differences discussed, site differences were documented that suggest differential use of OM based on site and watershed characteristics. Invertebrate and fish stable isotope signatures separated MR and CC samples. CC invertebrates were depleted in $\delta^{13}C$ relative to samples from MR, indicating invertebrates used more terrestrial-riverine OM at CC. Site differences among fish were variable by species. From the literature, we know that the use of OM by estuarine consumers varies by location and with river flow (Deegan and Garritt 1997; Darnaude et al. 2004b; Hoffman et al. 2007; Connolly et al. 2009). The results of this study

augment research showing that estuarine OM use is affected not only by river length and flow but also by watershed characteristics, such as glacial and forest coverage. The CC and MR watersheds differ in glacial and forest coverage as well as watershed area (Table 2.1). The increased forest coverage at CC may lead to higher export of terrestrial OM and, in turn, higher incorporation of this OM source into the diets of estuarine invertebrates. However, the site differences likely reflect the combined effect of multiple watershed features. Watershed features such as forest coverage, slope, and elevation change can all impact the type of material moving downstream at each site (Wipfli and Gregovich 2002; Wipfli 2005; Lisi et al. 2013). Research has shown that certain types of OM may be more beneficial to consumers and that consumers can preferentially take up specific OM sources when multiple sources are present (Martineau et al. 2004). Further investigation into the types and quality of OM available at each site may clarify species differences.

Despite differences among the sites studied, the contribution of allochthonous OM to marine fish diets was relatively low across sites. This finding and the uniting characteristics of each of the sites, including glacial influence and short system length, make these systems more similar to one another than other studied systems. The shared site characteristics may offer insight to how OM was used at these sites. Low incorporation of allochthonous OM even through sampling occurred in close proximity to river mouths, areas where the contribution of terrestrial OM is expected to be the highest (Deegan and Garritt 1997; Vinagre et al. 2008), may be in part related to discharge and circulation patterns that affect retention of OM in these estuaries. During higher flow periods such as those occurring during summer months (Weingartner et al. 2009), river flow can efficiently export OM out of the immediate vicinity of estuarine consumers (Peterson et al. 1994; Eyre 1998). The rapid export of OM and particulates

offshore has also been seen in highly channelized river systems (Syvitski et al. 2005; Howe 2012). For example, the north channel of the Skagit River in Puget Sound, WA, is highly channelized and research has shown that marsh OM contributes <10% to the diet of deposit feeding bivalves (*Macoma* spp.) in the estuary (Howe 2012). The short distance from headwaters to the estuary in our study systems may cause terrestrial-riverine OM to be transported in a manner resembling more developed, channelized systems.

The type and quality of the OM that is exported into the study estuaries may also have affected the observed OM use by estuarine consumers. While glacial rivers have been shown to transport large quantities of bioavailable DOC (Hood et al. 2009; Fellman et al. 2010), the export of macroinvertebrates and detritus can be highly variable among stream systems (Wipfli and Gregovich 2002). The high C:N ratio measured for POM samples collected from CC and MR suggest that river POM is predominately detrital and refractory material (Martineau et al. 2004) and may be of lower quality to consumers (Finlay and Kendall 2007). The quality of the OM exported to the marine environment is linked to the detrital conditioning of terrestrial material. As decaying leaf litter and detritus pass through wetlands, microbes colonize on the surface of the litter and break down cellulose making this material available to higher trophic levels (Boon et al. 2006; Spencer et al. 2007). The rapid transit times of these short systems and cold stream temperatures may limit the level of microbial conditioning of OM and in turn limit the use of allochthonous OM by estuarine consumers.

In addition to short river lengths, the lack of expansive wetlands ($\leq$ 5% watershed coverage) at each of the sites influences the strength of food web linkages. In systems with large wetlands and salt marshes, vascular plants, such as *Spartina*, can be a dominant carbon source for estuarine consumers (Peterson and Howarth 1987). For example, the Mattaponi River, a

tributary to the York River estuary in Virginia, is much longer than our study sites (166 km) and has extensive wetlands in the lower reaches (Virginia Department of Game and Inland Fisheries 2016). During periods of high freshwater discharge, greater than 65% of the diets of young-of-the-year shad in tidally-influenced reaches was composed of terrestrial OM (Hoffman et al. 2007). The limited wetlands at our study sites greatly reduces the potential OM contribution of this source, which allows a simplified two end member (terrestrial-riverine OM and marine OM) mixing model to be used. In this sense, our systems resemble mangrove and sea grass dominated systems where OM sources can be broken into two clear categories (Vaslet et al. 2012). Furthermore, the limited wetland habitat available to these sites and in turn the reduced contribution of a wetland OM source intermediate to our two end members may explain why sulfur stable isotopes were not required to separate our sources. Without wetland plants as a strong intermediate source, our terrestrial-riverine OM and marine OM sources could be separated by carbon and nitrogen alone.

Conclusions

In coastal SEAK, there is abundant terrestrial OM input to streams, with the potential for this material to be transported downstream into estuaries. Yet, our results indicate that estuarine consumers' use of terrestrial and riverine material is limited for marine fish but variable for anadromous fish and invertebrates. Differences among species, months, and sites underscores the reality that estuarine food web responses to allochthonous subsidies can be complex and dynamic. As glaciers in SEAK continue to thin and retreat (Larsen et al. 2007), the watershed characteristics of these study sites, such as glacial and forest coverage and river flow will change. Over longer time scales, reduced glacial coverage and increased forested areas may

result in greater incorporation of terrestrial-riverine OM in the diet of estuarine consumers. This study provides the opportunity to contrast current terrestrial-riverine OM use with use under future conditions.

Figures

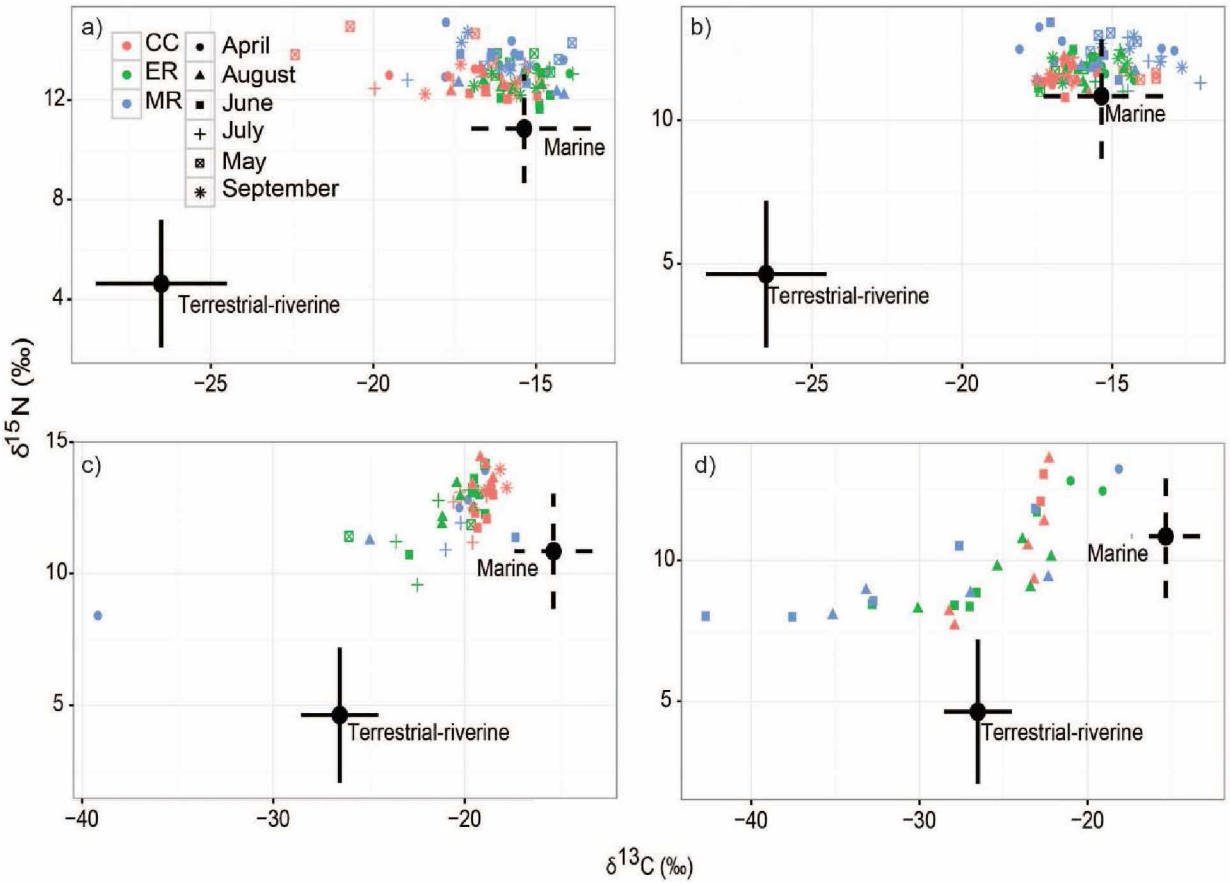


Fig. 2.1 Stable isotope biplots by fish species adjusted for trophic enrichment factors. Mean source values adjusted for trophic enrichment factors are shown by black points. Error bars indicate ±1 SD of the combined source and trophic enrichment factor SD. Staghorn sculpin (a), starry flounder (b), Dolly Varden (c), and coho salmon (d)

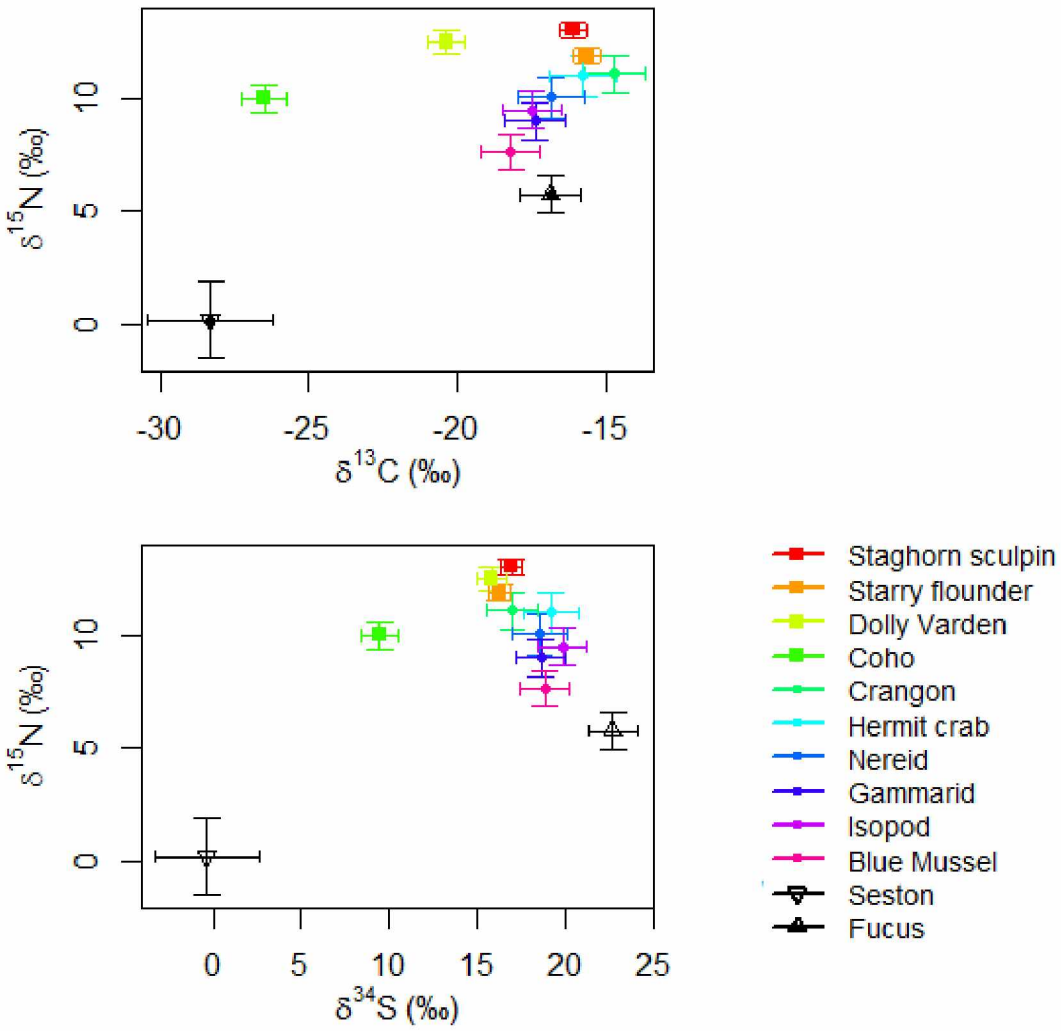


Fig. 2.2 Stable isotope biplots with source materials (triangles), invertebrates (circles), and fishes (squares). Raw values (not adjusted for trophic enrichment) were averaged across month and site. Error bars show the standard error for each isotope

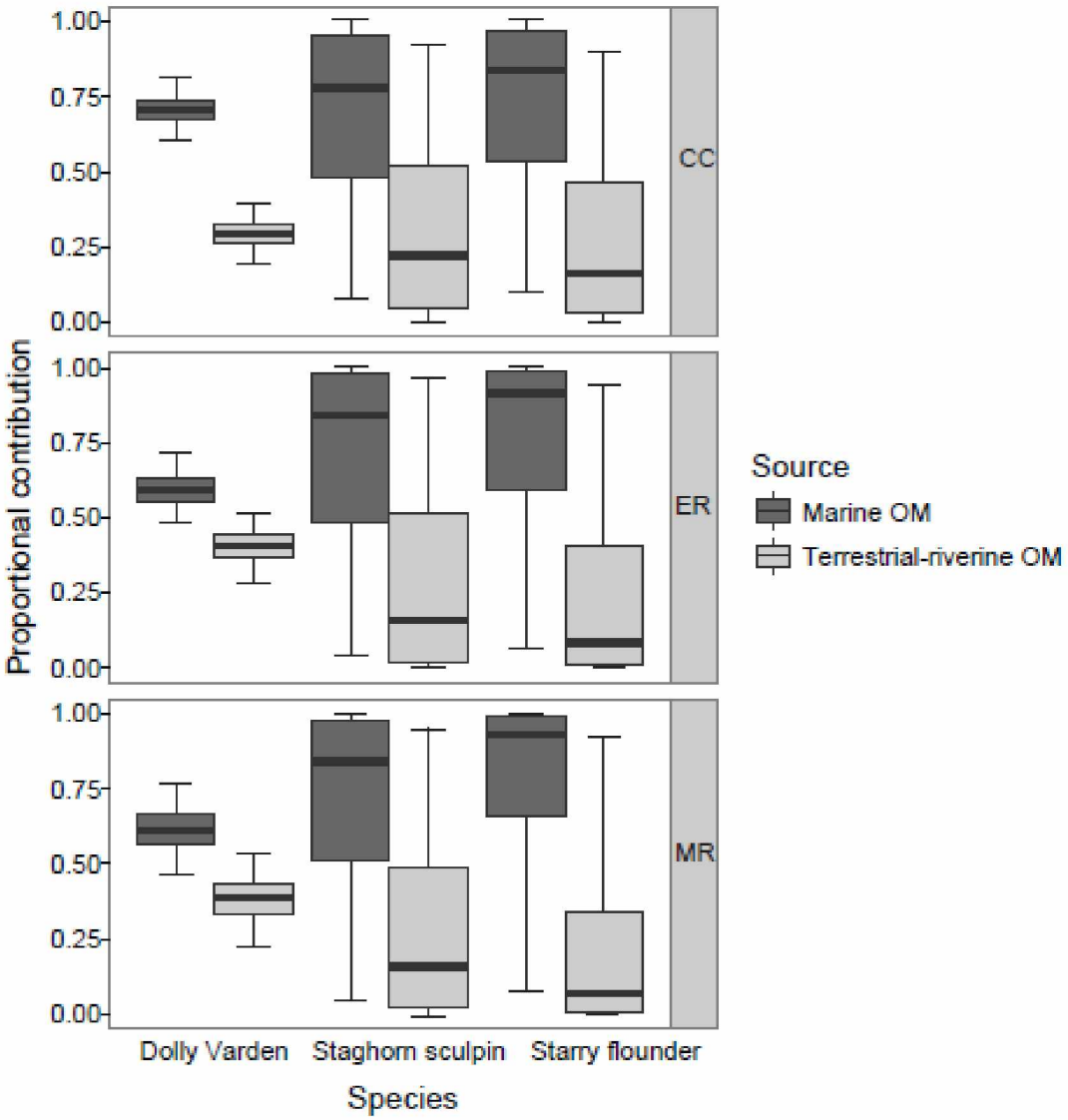


Fig. 2.3 Boxplots of the estimated proportions of terrestrial-riverine OM and marine OM in the diets of staghorn sculpin, starry flounder, and Dolly Varden, generated using Bayesian mixing models. The box length is the interquartile range with the median source contribution dissecting the box. The whiskers represent the 95% credible intervals

Tables

Table 2.1 Watershed characteristics for the Cowee Creek, Eagle River, and Mendenhall River watersheds

Site Characteristics	Cowee Creek	Eagle River[a]	Mendenhall River
Watershed area (km^2)[b]	110	276	222
River length (km)[c]	12.7	6.8-8.4	9.4
Glacier cover (%)[b]	13	48-49	63
Forest cover (%)[b]	57	23-25	8
Wetland coverage (%)[b]	5	2-5	1
Mean stream elevation (m)[b]	166	46-180	17
Mean stream slope (degrees)[b]	10	7-9	2.6
Mean estuary temperature (^0C)[d]	10.56	10.66	9.8
Mean estuary salinity (‰)[d]	19.14	20.82	16.69
Mean estuary turbidity (NTU)[d]	13.57	13.49	17.73

[a] The Herbert River joins the Eagle River approximately 1 km before the Eagle River estuary. The characteristics presented are for the combined Herbert and Eagle River watersheds.

[b] Fellman et al. (2014)

[c] USDAFS (2014)

[d] Data collected in this study, in conjunction with beach seining.

Table 2.2 Sample sizes by fish species, site, and month for stable isotope analysis

Species	Site	April	May	June	July	August	September	Total (n)
Staghorn sculpin	CC	5	5	5	5	5	5	30
	ER	5	5	5	5	5	5	30
	MR	5	5	5	5	5	5	30
Starry flounder	CC	5	5	5	5	5	5	30
	ER	5	5	5	5	5	5	30
	MR	5	5	5	5	5	5	30
Dolly Varden	CC	0	0	5	5	4	5	19
	ER	0	5	5	5	5	0	20
	MR	4	0	2	1	1	0	8
Coho	CC	0	2	6	0	0	0	8
	ER	0	5	5	2	0	0	12
	MR	0	5	4	1	0	0	10

Table 2.3 Stable isotope signatures of organic matter sources

Source	Marine OM (*Fucus* sp.) Mean ±SD	Terrestrial-riverine OM (POM) Mean ±SD
$\delta^{13}C$	-16.9 ±1.07 (n = 18)	-28.03 ±1.01 (n = 5)
$\delta^{15}N$	5.75 ±0.51 (n = 18)	0.47 ±1.43 (n = 5)
$\delta^{34}S$	22.73 ±0.16 (n = 18)	-1.17 ±4.61 (n = 3)

Table 2.4 Mean stable isotope values (‰) by species and site (CC: Cowee Creek estuary, ER: Eagle River estuary, MR: Mendenhall River estuary)

		$\delta^{13}C$			$\delta^{15}N$			$\delta^{34}S$		
Species	n	CC	ER	MR	CC	ER	MR	CC	ER	MR
Staghorn		-16.97	-15.37	-15.96	12.91	12.81	13.47	18.01	16.62	16.3
sculpin	90	(1.75)	(0.77)	(1.23)	(0.68)	(0.54)	(0.67)	(0.68)	(1.12)	(0.95)
Starry		-16.11	-15.72	-15.08	11.64	11.8	12.26	17.64	16.21	14.97
flounder	90	(1.07)	(0.92)	(1.54)	(0.38)	(0.44)	(0.61)	(0.38)	(1.13)	(1.08)
Dolly		-19.13	-20.61	-22.71	12.99	12.42	11.64	17.08	15.61	13.62
Varden	47	(0.72)	(1.89)	(7)	(0.8)	(1.09)	(1.63)	(1.11)	(2.25)	(2.86)
Coho		-24.14	-25.2	-29.95	10.73	9.92	9.54	13.04	9.17	6.9
salmon	30	(2.45)	(3.91)	(7.63)	(2.2)	(1.66)	(1.79)	(2.2)	(4.62)	(6.18)
Blue		-18.98	-18.2	-17.44	7.45	7.74	7.71	19.3	19.28	17.93
mussel	18	(0.48)	(0.56)	(0.45)	(0.48)	(0.23)	(0.31)	(0.48)	(0.44)	(0.58)
Isopod		-17.37	-18.41	-16.74	9.26	9.7	9.55	20.23	20.12	19.18
	18	(0.15)	(1.44)	(0.72)	(0.56)	(0.59)	(0.09)	(0.21)	(0.35)	(0.63)
Gammarid		-18.04	-17.65	-16.46	8.37	9.1	9.55	18.79	19.13	18.0
	18	(0.65)	(0.31)	(0.16)	(0.19)	(0.39)	(0.18)	(0.5)	(0.6)	(0.58)
Crangon		-14.84	-14.16	-14.99	11.07	10.98	11.21	17.97	16.44	16.26
shrimp	17	(0.46)	(0.93)	(0.91)	(0.38)	(0.73)	(0.54)	(0.49)	(2.16)	(0.64)
Hermit		-16.07	-16.27	-14.96	11.12	11.03	10.88	20.27	19.66	17.96
crab	14	(0.35)	(0.13)	(0.51)	(0.13)	(0.48)	(0.25)	(0.37)	(0.46)	(0.65)
Nereid		-17.84	-17.02	-13.35	9.0	10.82	10.92	20.05	18.38	14.58
	14	(1.06)	(0.56)	(0.37)	(0.6)	(0.61)	(0.92)	(0.25)	(0.95)	(1.01)

Table 2.5 Median proportions of terrestrial-riverine and marine OM in diets of invertebrates, estimated from Bayesian mixing models. The 95% credible intervals are shown in parentheses. The mean trophic enrichment factors (TEF) used in generating the estimates, with standard deviations in parentheses, are shown on the right

Median Source Proportion	Terrestrial/riverine OM			Marine OM			TEF	
Species	CC	ER	MR	CC	ER	MR	$\delta^{13}C$	$\delta^{15}N$
Blue mussel	0.267 (0.081-0.799)	0.201 (0.055-0.727)	0.155 (0.041-0.673)	0.733 (0.201-0.919)	0.799 (0.273-0.945)	0.845 (0.327-0.959)	1.1 (1.26)	2.8 (1.39)
Isopod	0.311 (0.023-0.943)	0.6 (0.072-0.984)	0.221 (0.01-0.925)	0.689 (0.057-0.977)	0.4 (0.016-0.928)	0.779 (0.75-0.99)	1.1 (1.26)	2.8 (1.39)
Gammarid	0.204 (0.076-0.805)	0.128 (0.037-0.693)	0.032 (0.004-0.384)	0.796 (0.195-0.924)	0.872 (0.307-0.963)	0.968 (0.616-0.996)	1.3 (1.95)	3.45 (2.79)
Crangon shrimp	0.402 (0.014-0.951)	0.365 (0.005-0.976)	0.383 (0.005-0.98)	0.598 (0.049-0.86)	0.635 (0.024-0.995)	0.617 (0.02-0.995)	1.5 (1.74)	5.1 (2.13)
Hermit crab	0.396 (0.011-0.95)	0.39 (0.005-0.978)	0.32 (0.005-0.978)	0.604 (0.05-0.989)	0.61 (0.022-0.995)	0.68 (0.022-0.995)	1.5 (1.74)	5.1 (2.13)
Nereid	0.435 (0.011-0.955)	0.399 (0.005-0.984)	0.398 (0.005-0.982)	0.565 (0.045-0.989)	0.601 (0.016-0.995)	0.602 (0.018-0.995)	1.5 (1.74)	5.1 (2.13)

References

Abrantes, K. G., A. Barnett, T. R. Marwick, and S. Bouillon. 2013. Importance of terrestrial subsidies for estuarine food webs in contrasting East African catchments. *Ecosphere* 4: 1–33.

Arimitsu, M. L. 2016. Influence of glaciers on coastal marine ecosystems in the Gulf of Alaska. University of Alaska Fairbanks.

Bell, L. E., B. A. Bluhm, and K. Iken. 2016. Influence of terrestrial organic matter in marine food webs of the Beaufort Sea shelf and slope. *Marine Ecology Progress Series* 550: 1–24. doi:10.3354/meps11725.

Boon, P. I., P. C. Pollard, and D. Ryder. 2006. Wetland microbial ecology and biogeochemistry. In *Ecology of Freshwater and Estuarine Wetlands*, ed. D. P. Batzer and R. R. Sharitz. Berkeley: University of California Press.

Bouillon, S., P. Chandra Mohan, N. Sreenivas, and F. Dehairs. 2000. Sources of suspended organic matter and selective feeding by zooplankton in an estuarine mangrove ecosystem as traced by stable isotopes. *Marine Ecology Progress Series* 208: 79–92. doi:10.3354/meps208079.

Brittain, J. E., and A. M. Milner. 2001. Ecology of glacier-fed rivers: current status and concepts. *Freshwater Biology* 46: 1571–1578. doi:10.1046/j.1365-2427.2001.00845.x.

Church, M. R., J. L. Ebersole, K. M. Rensmeyer, R. B. Couture, F. T. Barrows, and D. L.G. Noakes. 2009. Mucus: a new tissue fraction for rapid determination of fish diet switching using stable isotope analysis. *Canadian Journal of Fisheries and Aquatic Sciences* 66: 1–5. doi:10.1139/F08-206.

City and Borough of Juneau (CBJ). 2016. Juneau trails project from the City and Bureau of Juneau: Cowee-Davies.

Clarke, K. R., and R. N. Gorley. 2015. PRIMER v7 : User manual/tutorial. Plymouth: PRIMER-E.

Clarke, K. R., R. N. Gorley, P. J. Somerfield, and R. M. Warwick. 2014. Change in marine communities: an approach to statistical analysis and interpretation. Plymouth: PRIMER-E.

Connolly, R. M., M. A. Guest, A. J. Melville, and J. M. Oakes. 2004. Sulfur stable isotopes separate producers in marine food-web analysis. *Oecologia* 138: 161–7. doi:10.1007/s00442-003-1415-0.

Connolly, R. M., T. A. Schlacher, and T. F. Gaston. 2009. Stable isotope evidence for trophic subsidy of coastal benthic fisheries by river discharge plumes off small estuaries. *Marine Biology Research* 5: 164–171. doi:10.1080/17451000802266625.

Creque, S. M., and S. J. Czesny. 2012. Diet overlap of non-native alewife with native yellow perch and spottail shiner in nearshore waters of southwestern Lake Michigan, 2000-2007. *Ecology of Freshwater Fish* 21: 207–221. doi:10.1111/j.1600-0633.2011.00538.x.

Darnaude, A. M., C. Salen-Picard, and M. L. Harmelin-Vivien. 2004a. Depth variation in terrestrial particulate organic matter exploitation by marine coastal benthic communities off the Rhone River delta (NW Mediterranean). *Marine Ecology Progress Series* 275: 47–57. doi:10.3354/meps275047.

Darnaude, A. M., C. Salen-Picard, N. V. C. Polunin, and M. L. Harmelin-Vivien. 2004b. Trophodynamic linkage between river runoff and coastal fishery yield elucidated by stable isotope data in the Gulf of Lions (NW Mediterranean). *Oecologia* 138: 325–332. doi:10.1007/s00442-003-1457-3.

Deegan, L. A., and R. H. Garritt. 1997. Evidence for spatial variability in estuarine food webs. *Marine Ecology Progress Series* 147: 31–47. doi:10.3354/meps147031.

DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42: 495–506. doi:10.1016/0016-7037(78)90199-0.

Eyre, B. 1998. Transport, retention and transformation of material in Australian estuaries. *Estuaries* 21: 540. doi:10.2307/1353293.

Fellman, J. B., E. Hood, P. A. Raymond, J. Hudson, M. Bozeman, and M. Arimitsu. 2015. Evidence for the assimilation of ancient glacier organic carbon in a proglacial stream food web. *Limnology and Oceanography* 60: 1118–1128. doi:10.1002/lno.10088.

Fellman, J. B., S. Nagorski, S. Pyare, A. W. Vermilyea, D. Scott, and E. Hood. 2014. Stream temperature response to variable glacier coverage in coastal watersheds of Southeast Alaska. *Hydrological Processes* 28: 2062–2073. doi:10.1002/hyp.9742.

Fellman, J. B., R. G. M. Spencer, P. J. Hernes, R. T. Edwards, D. V. D'Amore, and E. Hood. 2010. The impact of glacier runoff on the biodegradability and biochemical composition of terrigenous dissolved organic matter in near-shore marine ecosystems. *Marine Chemistry* 121: 112–122. doi:10.1016/j.marchem.2010.03.009.

Finlay, J. C. 2001. Stable-carbon-isotope ratios of river biota: implications for energy flow in lotic food webs. *Ecology* 82: 1052–1064.

Finlay, J. C., and C. Kendall. 2007. Stable isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems. In *Stable Isotopes in Ecology and Environmental Science*, ed. R. H. Michener and K. Lajtha, 2nd ed., 283–333. Malden: Blackwell Publishing.

Forbes, D. L. 2011. Glaciated Coasts. In *Treatise on Estuarine and Coastal Science*, ed. E. Wolanski and D. S. McLusky, 223–243. San Diego: Academic Press.

Franca, S., R. P. Vasconcelos, S. Tanner, C. Maguas, M. J. Costa, and H. N. Cabral. 2011. Assessing food web dynamics and relative importance of organic matter sources for fish species in two Portuguese estuaries: a stable isotope approach. *Marine Environmental Research* 72: 204–215. doi:10.1016/j.marenvres.2011.09.001.

Fry, B. 2002. Conservative mixing of stable isotopes across estuarine salinity gradients: A conceptual framework for monitoring watershed influences on downstream fisheries production. *Estuaries* 25: 264–271. doi:10.1007/BF02691313.

Gelman, A., and D. B. Rubin. 1992. Inference from iterative simulation using multiple sequences. *Statistical Science* 7: 457–511.

Goering, J., V. Alexander, and N. Haubenstock. 1990. Seasonal variability of stable carbon and nitrogen isotope ratios of organisms in a North Pacific Bay. *Estuarine, Coastal and Shelf Science* 30: 239–260.

Heady, W. N., and J. W. Moore. 2013. Tissue turnover and stable isotope clocks to quantify resource shifts in anadromous rainbow trout. *Oecologia* 172: 21–34. doi:10.1007/s00442-012-2483-9.

Hill, D. F., N. Bruhis, S. E. Calos, A. Arendt, and J. Beamer. 2015. Spatial and temporal variability of freshwater discharge into the Gulf of Alaska. *Journal of Geophysical Research: Oceans* 120: 634–646. doi:10.1002/2014JC010395.

Hill, J. M., and C. D. McQuaid. 2011. Stable isotope methods: The effect of gut contents on isotopic ratios of zooplankton. *Estuarine, Coastal and Shelf Science* 92: 480–485. doi:10.1016/j.ecss.2011.02.002.

Hobson, K. A. 1990. Stable isotope analysis of Marbled Murrelets: evidence for freshwater feeding and determination of trophic level. *Condor* 92: 897–903.

Hobson, K. A. 1999. Tracing origins and migration of wildlife using stable isotopes : a review. *Oecologia* 120: 314–326.

Hoffman, J. C., D. A. Bronk, and J. E. Onley. 2007. Contribution of allochthonous carbon to American shad production in the Mattaponi River, Virginia, using stable isotopes. *Estuaries and Coasts* 30: 1034–1048.

Hood, E., and L. Berner. 2009. Effects of changing glacial coverage on the physical and biogeochemical properties of coastal streams in southeastern Alaska. *Journal of Geophysical Research* 114. doi:10.1029/2009jg000971.

Hood, E., J. Fellman, R. G. M. Spencer, P. J. Hernes, R. Edwards, D. D'Amore, and D. Scott. 2009. Glaciers as a source of ancient and labile organic matter to the marine environment. *Nature* 462. Nature Publishing Group: 1044–7. doi:10.1038/nature08580.

Howe, E. R. 2012. Detrital shadows: Evaluating landscape and species effects on detritus-based food web connectivity in Pacific Northwest estuaries. University of Washington.

Ingram, T., and B. Matthews. 2007. Lipid extraction has little effect on the $\delta^{15}N$ of aquatic consumers. *Limnology and Oceanography: Methods*: 338–343.

Jaecks, T., and T. P. Quinn. 2014. Ontogenetic shift to dependence on salmon-derived nutrients in Dolly Varden char from the Iliamna River, Alaska. *Environmental Biology of Fishes* 97: 1323–1333. doi:10.1007/s10641-014-0221-3.

Larsen, C. F., R. J. Motyka, A. A. Arendt, K. A. Echelmeyer, and P. E. Geissler. 2007. Glacier changes in southeast Alaska and northwest British Columbia and contribution to sea level rise. *Journal of Geophysical Research* 112: F01007. doi:10.1029/2006JF000586.

Lindeberg, M. R., and S. C. Lindstrom. 2010. *Field guide to seaweeds of Alaska*. Fairbanks: Alaska Sea Grant.

Lisi, P. J., D. E. Schindler, K. T. Bentley, and G. R. Pess. 2013. Association between geomorphic attributes of watersheds, water temperature, and salmon spawn timing in Alaskan streams. *Geomorphology* 185. Elsevier B.V.: 78–86. doi:10.1016/j.geomorph.2012.12.013.

Martineau, C., W. F. Vincent, J. J. Frenette, and J. J. Dodson. 2004. Primary consumers and particulate organic matter: Isotopic evidence of strong selectivity in the estuarine transition zone. *Limnology and Oceanography* 49: 1679–1686. doi:10.4319/lo.2004.49.5.1679.

Marty, J., and D. Planas. 2008. Comparison of methods to determine algal $\delta^{13}C$ in freshwater. *Limnology and Oceanography: Methods* 6: 51–63.

McCutchan, J. H., W. M. Lewis, C. Kendall, and C. C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 2: 378–390.

Michener, R. H., and L. Kaufman. 2007. Stable isotope ratios as tracers in marine food webs: An update. In *Stable Isotopes in Ecology and Environmental Science*, ed. R. H. Michener and K. Lajtha, 2nd ed., 239–282. Malden: Blackwell Publishing.

Milner, A. M., and G. E. Petts. 1994. Glacial rivers: physical habitat and ecology. *Freshwater Biology* 32: 295–307. doi:10.1111/j.1365-2427.1994.tb01127.x.

Montoya, J. P. 2007. Natural abundance of 15N in marine planktonic ecosystems. In *Stable Isotopes in Ecology and Environmental Science*, ed. R. H. Michener and K. Lajtha, 2nd ed., 176–201. Malden: Blackwell Publishing.

Moore, J. W., and B. X. Semmens. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. *Ecology Letters* 11: 470–480. doi:10.1111/j.1461-0248.2008.01163.x.

National Weather Service. 2014. Climate Report National Weather Service Juneau. Juneau: U.S. Department of Commerce, NOAA.

Neal, E. G., E. Hood, and K. Smikrud. 2010. Contribution of glacier runoff to freshwater discharge into the Gulf of Alaska. *Geophysical Research Letters* 37: L06404. doi:10.1029/2010gl042385.

Nixon, S. W., C. A. Oviatta, J. Frithsena, and B. Sullivana. 1986. Nutrients and the productivity of estuarine and coastal marine ecosystems. *Journal of the Limnological Society of Southern Africa* 12: 43–71.

O'Neel, S., E. Hood, A. L. Bidlack, S. W. Fleming, M. L. Arimitsu, A. Arendt, E. Burgess, et al. 2015. Icefield-to-ocean linkages across the northern Pacific coastal temperate rainforest ecosystem. *BioScience* 65: 499–512. doi:10.1093/biosci/biv027.

Pasquaud, S., J. Lobry, and P. Elie. 2007. Facing the necessity of describing estuarine ecosystems: a review of food web ecology study techniques. *Hydrobiologia* 588: 159–172. doi:10.1007/s10750-007-0660-3.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. *Annual Reivew of Ecology and Systematics* 18: 293–320.

Peterson, B. J., B. Fry, M. Hullar, S. Saupe, and R. Wright. 1994. The distribution and stable carbon isotopic composition of dissolved organic carbon in estuaries. *Estuaries* 17: 111–121.

Peterson, B. J., and R. W. Howarth. 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. *Limnology and Oceanography* 32: 1195–1213. doi:10.4319/lo.1987.32.6.1195.

Peterson, B. J., R. W. Howarth, and R. H. Garritt. 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science* 227. American Association for the Advancement of Science: 1361–1363. doi:10.2307/1695096.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annual Reivew of Ecology and Systematics* 28: 289–316.

Semmens, B. X., E. J. Ward, J. W. Moore, and C. T. Darimont. 2009. Quantifying inter-and intra-population niche variability using hierarchical bayesian stable isotope mixing models. *PLoS ONE* 4: 1–9. doi:10.1371/journal.pone.0006187.

Søreide, J. E., T. Tamelander, H. Hop, K. A. Hobson, and I. Johansen. 2006. Sample preparation effects on stable C and N isotope values: a comparison of methods in Arctic marine food web studies. *Marine Ecology Progress Series* 328: 17–28. doi:10.3354/meps328017.

Spencer, R. G. M., J. M. E. Ahad, A. Baker, G. L. Cowie, R. Ganeshram, R. C. Upstill-Goddard, and G. Uher. 2007. The estuarine mixing behaviour of peatland derived dissolved organic carbon and its relationship to chromophoric dissolved organic matter in two North Sea estuaries (U.K.). *Estuarine, Coastal and Shelf Science* 74: 131–144. doi:10.1016/j.ecss.2007.03.032.

Spiker, E. C., and L. E. Schemel. 1979. Distribution and stable-isotope composition of carbon in San Francisco Bay. In *San Francisco Bay: The urbanized estuary*, ed. T. J. Conomos, 195–212. San Francisco: Pacific Division, American Association for the Advancement of Science.

Stock, B., and B. Semmens. 2015. MixSIAR User Manual v3.0. https://github.com/brianstock/MixSIAR. doi:10.5281/zenodo.47719.

Syvitski, J. P. M., A. J. Kettner, A. Correggiari, and B. W. Nelson. 2005. Distributary channels and their impact on sediment dispersal. *Marine Geology* 222-223: 75–94. doi:10.1016/j.margeo.2005.06.030.

Szabo, A., A Tudge, J. Macnamara, and H.G. Thode. 1950. Distribution of S34 in nature and the sulfur Cycle. *Science* 111: 464–465.

U.S. Department of Agriculture Forest Service (USDAFS) Tongass National Forest. 2014. AK Hydro Streams in Southeast Alaska. Southeast Alaska GIS Library.

Vander Zanden, M. J., and J. B. Rasmussen. 2001. Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography* 46: 2061–2066. doi:10.4319/lo.2001.46.8.2061.

Vaslet, A., D. L. Phillips, C. France, I. C. Feller, and C. C. Baldwin. 2012. The relative importance of mangroves and seagrass beds as feeding areas for resident and transient fishes among different mangrove habitats in Florida and Belize: Evidence from dietary and stable-isotope analyses. *Journal of Experimental Marine Biology and Ecology* 434-435. Elsevier B.V.: 81–93. doi:10.1016/j.jembe.2012.07.024.

Vinagre, C., J. Salgado, M. J. Costa, and H. N. Cabral. 2008. Nursery fidelity, food web interactions and primary sources of nutrition of the juveniles of *Solea solea* and *S. senegalensis* in the Tagus estuary (Portugal): A stable isotope approach. *Estuarine, Coastal and Shelf Science* 76: 255–264. doi:10.1016/j.ecss.2007.07.006.

Virginia Department of Game and Inland Fisheries. 2016. Mattaponi River.

Weingartner, T., L. Eisner, G. L. Eckert, and S. Danielson. 2009. Southeast Alaska: oceanographic habitats and linkages. *Journal of Biogeography* 36: 387–400. doi:10.1111/j.1365-2699.2008.01994.x.

Whitney, E. J. 2016. Trophic Ecology of Nearshore Fishes in Glacially-Influenced Estuaries of Southeast Alaska. University of Alaska Fairbanks.

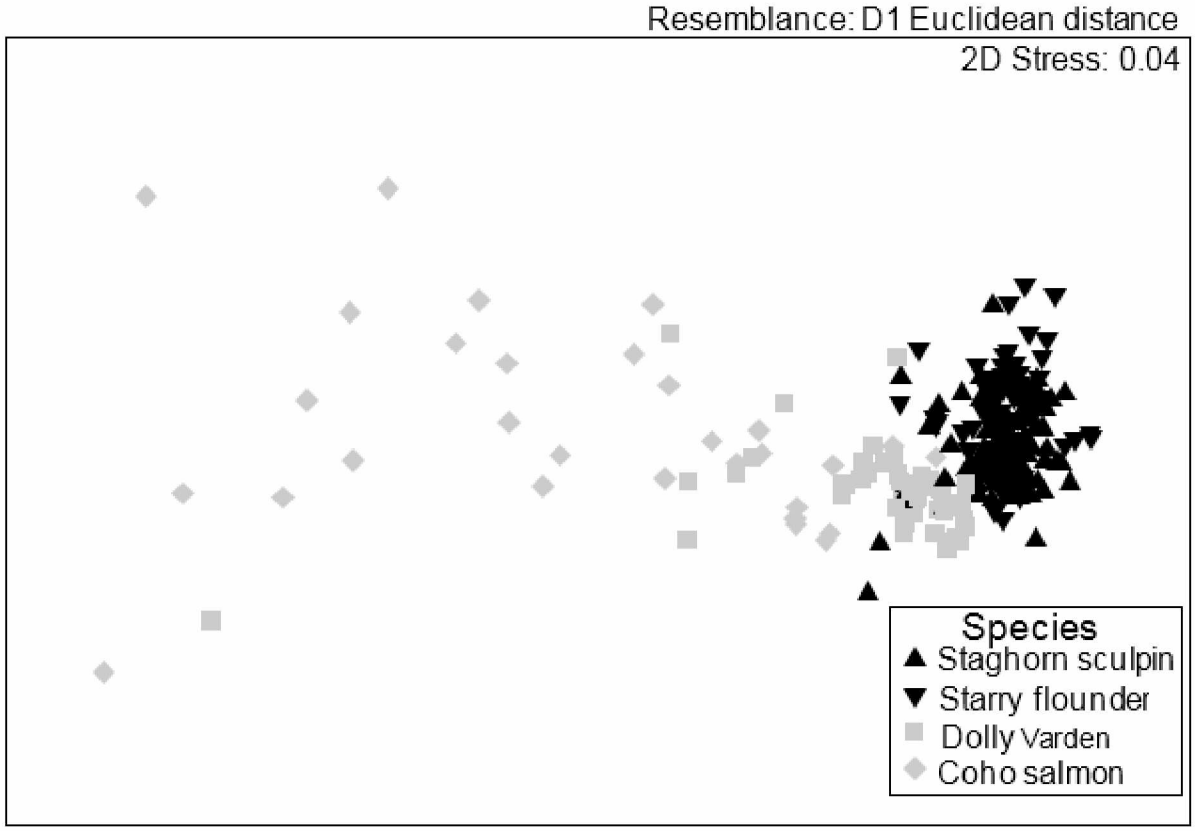
Wipfli, M. S. 2005. Trophic linkages between headwater forests and downstream fish habitats: implications for forest and fish management. *Landscape and Urban Planning* 72: 205–213. doi:10.1016/j.landurbplan.2004.09.025.

Wipfli, M. S., and D. P. Gregovich. 2002. Export of invertebrates and detritus from fishless headwater streams in southeastern Alaska: implications for downstream salmonid production. *Freshwater Biology* 47: 957–969. doi:10.1046/j.1365-2427.2002.00826.x.

Wolanski, E., and D. S. McLusky, ed. 2011. *Treatise on Estuarine and Coastal Science*. San Diego: Academic Press.

Appendix

Appendix 2.A Principal component analysis plot of fish stable isotope signatures showing separation of marine (staghorn sculpin and starry flounder; black triangles) and anadromous (Dolly Varden and coho salmon; gray rectangles) species



General Conclusions

Diet composition of staghorn sculpin

Staghorn sculpins were the focus of chapter one not only because they were abundant in the catch across sites but also because they have shown dietary responses to changes in prey availability (Tasto 1975; Mace 1983; Armstrong et al. 1995) and, therefore, could be a potential indicator of changes in their feeding environment. The staghorn sculpins sampled consumed a wide variety of prey items, including 81 taxa of invertebrate and vertebrate prey. Four epibenthic invertebrate taxa (gammarids, mysids, isopods, and polychaetes) occurred most frequently in diets across sites and months and composed over 47% by weight of the diets examined. Other diet studies of staghorn sculpin from the Tijuana Estuary, California, to Vancouver Island, British Columbia, have also documented use of similar dominant prey taxa (Tasto 1975; Mace 1983; Armstrong et al. 1995; West et al. 2003). Although they represented less than 1% by weight of staghorn sculpin diets, prey of terrestrial and freshwater origin were also found in stomachs across sampling months at a low frequency of occurrence (8.7% FO). Teleost prey consumption (34% W) reflected the seasonal presence of juvenile fish in the estuary. Fish consumption peaked in June, when juvenile salmon were most abundant in our beach seine catches. The generalist feeding behavior of staghorn sculpins, including their consistent use of dominant prey taxa across sampling periods and sites, combined with their predation on freshwater tolerant and terrestrial prey species indicate that staghorn sculpin may be resilient to environmental change associated with increased glacial meltwater.

Spatial and temporal variation in the diets of staghorn sculpin

Diet composition showed weak to moderate differences across sampling periods with variable river flow and sites with differing watershed characteristics, as demonstrated by an analysis of similarity (ANOSIM). These differences reflect the combined effect of seasonal and site variation in freshwater flow as well as the phenology of estuarine prey, such as the June peak in juvenile salmon abundance. There is evidence that both of these factors contribute to dietary differences, but our ability to separate the effects of freshwater flow and phenology of prey is limited by a single year of data collection.

Climate change models for Southeast Alaska (SEAK) forecast increased temperatures and precipitation, which together will increase freshwater runoff into estuaries (SNAP 2008, McAfee et al. 2014). These climate-induced changes in estuarine environments will likely alter the diet composition of staghorn sculpins. Estuarine conditions may become inhospitable to certain prey taxa, particularly obligate marine species, due to the increase of freshwater. For instance, snake prickleback (*Lumpenus sagitta*), could occur less frequently in the diet of staghorn sculpin, as this species has been shown to be negatively affected by low salinity conditions (Tresierra-Aguilar 1980). Additionally, in lower salinity conditions, *Gnorimosphaeroma oregonensis* in the diet of staghorn sculpin may be replaced by *G. insulare*, the more freshwater tolerant species (Kozloff 1996). This study takes a first step toward understanding how seasonal variation in freshwater runoff alters the feeding ecology of an estuarine predator. The data collected in this project could be valuable in assessing shifts in diet composition and the frequency of occurrence of prey taxa in diets of staghorn sculpin when compared with diet data collected under contrasting estuarine conditions. Future studies could be conducted during summer periods of high freshwater discharge, which climate forecasts predict

are likely to occur, but sampling of staghorn sculpin diets during winter months would also be informative. Freshwater discharge from glacial rivers decreases during winter months; therefore, comparing staghorn sculpin diets across seasons may be valuable in understanding the impact of freshwater runoff.

Use of allochthonous OM by estuarine consumers

Diet and stable isotope analyses are complementary approaches that inform our understanding of how the environments adjacent to estuaries interact and function across space and time from a food web perspective (Pasquaud et al. 2007). In the second chapter, we analyzed stable isotope signatures of estuarine consumers across sites and months of variable freshwater flow. Stable isotope analyses of estuarine fish showed limited temporal and spatial variability in OM use. Marine fishes indicated heavy reliance on marine OM sources and anadromous fishes incorporated more terrestrial-riverine OM. From the literature, we know that the use of OM by estuarine consumers varies by location as well as with river flow (Deegan and Garritt 1997; Darnaude et al. 2004; Hoffman et al. 2007; Connolly et al. 2009). The results of this study augment research showing that OM use in estuaries is affected not only by freshwater discharge but also by watershed characteristics, such as glacial and forest coverage.

A multispecies approach is needed to understand OM use in estuaries, as shown by the differences in OM use among consumers. We found limited use of terrestrial-riverine OM by marine fishes (7-22% terrestrial-riverine OM) and only slightly greater use by intertidal invertebrates (3-60% terrestrial-riverine OM). Anadromous fishes showed higher terrestrial-riverine OM use and greater individual variation compared to marine fishes, reflecting differences in foraging, with some individuals incorporating a higher percentage of

allochthonous OM than others do. The tight grouping of staghorn sculpin stable isotope signatures is consistent with the limited individual variation observed in the diets, indicated by the high frequency of occurrence of dominant prey items.

Site characteristics contributing to allochthonous OM use

Given the potentially high flux of terrestrial and freshwater OM associated with high freshwater discharge rates to SEAK estuaries, we hypothesized that allochthonous OM would provide a sizable subsidy to estuarine consumers. In the record-setting wet summer of 2014 (National Weather Service 2014), however, the estuarine consumers sampled showed a lower reliance on allochthonous OM than expected. Though less marked than species differences, site differences may give insight as to why allochthonous OM was not highly utilized by estuarine consumers despite its prevalence (Hood et al. 2009; Fellman et al. 2015). Invertebrates from estuaries adjacent to Cowee Creek and Mendenhall River showed strong differences in stable isotope signatures. Cowee Creek estuary invertebrate samples were depleted in $\delta^{13}C$ and $\delta^{15}N$ compared to those from the Mendenhall River estuary, reflecting an increased use of terrestrial and riverine OM at the Cowee Creek site. Cowee Creek has lower glacial and higher forest coverage in the watershed (Fellman et al. 2014) and more low gradient floodplain channels than either of the other two sites (CBJ 2016). This suggests that characteristics of glacial watersheds may limit terrestrial and riverine OM use by estuarine consumers.

Glacial coverage in the watersheds and the short length of rivers flowing into SEAK estuaries sets our study system apart from estuaries in other parts of the world. All of the sites studied are short systems with only a few kilometers between the glacial headwaters and the estuaries. Short transit times to estuaries and the lack of expansive wetlands ($\leq$ 5% watershed

coverage) may limit the time for microbial conditioning of OM and in turn limit the use of allochthonous OM by estuarine consumers. As decaying leaf litter and detritus pass through wetlands microbes play a role in conditioning these OM sources and making them available to higher trophic levels (Boon et al. 2006; Spencer et al. 2007). In addition, in other study systems, wetland vascular plants, such as *Spartina*, can be a dominant carbon source for estuarine consumers (Peterson and Howarth 1987), which is minimally available in sites with limited wetland coverage. Thus, glacially-influenced streams may function differently than longer, clearwater streams that provide increased opportunity for microbial conditioning in wetlands. For example, the Mattaponi River, a tributary to the York River estuary in Virginia, is much longer than our study sites (166 km) and has extensive wetlands in the lower reaches (Virginia Department of Game and Inland Fisheries 2016). During periods of high freshwater discharge, greater than 65% of the diets of young-of-the-year shad in tidally-influenced reaches was composed of terrestrial OM (Hoffman et al. 2007).

The differences between glacially-influenced and clearwater streams may change over time with forecasted climate changes. In the near-term, with the continued input of cold, turbid, freshwater from melting glaciers, estuarine consumers will continue to be supported primarily by marine-derived OM. Over longer time scales when the glaciers have largely melted and glacial influence on estuary conditions is reduced, we may see greater incorporation of terrestrial and riverine OM in the diets of estuarine consumers as the adjacent watersheds transition from glaciated to forested. The greater use of terrestrial and riverine OM by invertebrates at Cowee Creek compared to the more glacially-influenced Mendenhall River supports this prediction. Increased forest coverage may increase the flux of OM from the terrestrial environment and warmer river water temperatures may allow for increased microbial processing of detritus, which

together support increased use of allochthonous OM by estuarine consumers. While these estuaries will continue to be fed by short river systems, the trophic linkages between the adjacent habitats may shift with the continued flux of freshwater from melting glaciers.

References

Arendt, A. A., K. A. Echelmeyer, and W. D. Harrison. 2002. Rapid wastage of Alaska glaciers and their contribution to rising sea level. *Science* 297: 382–386.

Arimitsu, M. L., M. A. Litzow, J. F. Piatt, M. D. Robards, A. A. Abookire, and G. S. Drew. 2003. *Inventory of marine and estuarine fishes in Southeast and Central Alaska National Parks.* Nat. Park. Serv. Alaska Region. Inventory and Monitoring Program Final Rep., USGS Alaska Science Center, Anchorage Alaska. 79 p.

Armstrong, J. L., D. A. Armstrong, and S. B. Mathews. 1995. Food habits of estuarine staghorn sculpin, *Leptocottus armatus* with focus on consumption of juvenile Dungeness crab *Cancer magister*. *Fisheries Research* 93: 456–470.

Baker, L., K. Koski, D. Albert, and N. Cohen. 2011. *A Conservation Action Plan for Estuarine Ecosystems of Southeastern Alaska.*

Boon, P. I., P. C. Pollard, and D. Ryder. 2006. Wetland microbial ecology and biogeochemistry. In *Ecology of Freshwater and Estuarine Wetlands*, ed. D. P. Batzer and R. R. Sharitz. Berkeley: University of California Press.

City and Borough of Juneau (CBJ). 2016. Juneau trails project from the City and Bureau of Juneau: Cowee-Davies.

Connolly, R. M., T. A. Schlacher, and T. F. Gaston. 2009. Stable isotope evidence for trophic subsidy of coastal benthic fisheries by river discharge plumes off small estuaries. *Marine Biology Research* 5: 164–171. doi:10.1080/17451000802266625.

Darnaude, A. M., C. Salen-Picard, N. V. C. Polunin, and M. L. Harmelin-Vivien. 2004. Trophodynamic linkage between river runoff and coastal fishery yield elucidated by stable isotope data in the Gulf of Lions (NW Mediterranean). *Oecologia* 138: 325–332. doi:10.1007/s00442-003-1457-3.

Deegan, L. A., and R. H. Garritt. 1997. Evidence for spatial variability in estuarine food webs. *Marine Ecology Progress Series* 147: 31–47. doi:10.3354/meps147031.

Fellman, J. B., E. Hood, P. A. Raymond, J. Hudson, M. Bozeman, and M. Arimitsu. 2015. Evidence for the assimilation of ancient glacier organic carbon in a proglacial stream food web. *Limnology and Oceanography* 60: 1118–1128. doi:10.1002/lno.10088.

Fellman, J. B., S. Nagorski, S. Pyare, A. W. Vermilyea, D. Scott, and E. Hood. 2014. Stream temperature response to variable glacier coverage in coastal watersheds of Southeast Alaska. *Hydrological Processes* 28: 2062–2073. doi:10.1002/hyp.9742.

Fellman, J. B., R. G. M. Spencer, P. J. Hernes, R. T. Edwards, D. V. D'Amore, and E. Hood. 2010. The impact of glacier runoff on the biodegradability and biochemical composition of terrigenous dissolved organic matter in near-shore marine ecosystems. *Marine Chemistry* 121: 112–122. doi:10.1016/j.marchem.2010.03.009.

Forbes, D. L. 2011. Glaciated Coasts. In *Treatise on Estuarine and Coastal Science*, ed. E. Wolanski and D. S. McLusky, 223–243. San Diego: Academic Press.

Hill, D. F., N. Bruhis, S. E. Calos, A. Arendt, and J. Beamer. 2015. Spatial and temporal variability of freshwater discharge into the Gulf of Alaska. *Journal of Geophysical Research: Oceans* 120: 634–646. doi:10.1002/2014JC010395.

Hoffman, J. C., D. A. Bronk, and J. E. Onley. 2007. Contribution of allochthonous carbon to American shad production in the Mattaponi River, Virginia, using stable isotopes. *Estuaries and Coasts* 30: 1034–1048.

Hood, E., and L. Berner. 2009. Effects of changing glacial coverage on the physical and biogeochemical properties of coastal streams in southeastern Alaska. *Journal of Geophysical Research* 114. doi:10.1029/2009jg000971.

Hood, E., J. Fellman, R. G. M. Spencer, P. J. Hernes, R. Edwards, D. D'Amore, and D. Scott. 2009. Glaciers as a source of ancient and labile organic matter to the marine environment. *Nature* 462. Nature Publishing Group: 1044–7. doi:10.1038/nature08580.

Kostecki, C., F. Le Loc'h, J. M. Roussel, N. Desroy, D. Huteau, P. Riera, H. Le Bris, and O. Le Pape. 2010. Dynamics of an estuarine nursery ground: the spatio-temporal relationship between the river flow and the food web of the juvenile common sole (*Solea solea*, L.) as revealed by stable isotopes analysis. *Journal of Sea Research* 64. Elsevier B.V.: 54–60. doi:10.1016/j.seares.2009.07.006.

Kozloff, E. N. 1996. *Marine invertebrates of the Pacific Northwest.* Seattle: University of Washington Press.

Larsen, C. F., R. J. Motyka, A. A. Arendt, K. A. Echelmeyer, and P. E. Geissler. 2007. Glacier changes in southeast Alaska and northwest British Columbia and contribution to sea level rise. *Journal of Geophysical Research* 112: F01007. doi:10.1029/2006JF000586.

Mace, P. M. 1983. Predator-prey funcational responses and predation by staghorn sculpin (*Leptocottus armatus*) on Chum Salmon Fry (*Onchorhynchus keta*). University of British Columbia. doi:10.1017/CBO9781107415324.004.

McAfee, S. A., J. Walsh, and T. S. Rupp. 2014. Statistically downscaled projections of snow/rain partitioning for Alaska. *Hydrological Processes* 28: 3930–3946. doi:10.1002/hyp.9934.

Mecklenburg, C. W., T. A. Mecklenburg, and L. K. Thorsteinson. 2002. *Fishes of Alaska.* Bethesda: American Fisheries Society.

National Weather Service. 2014. Climate Report National Weather Service Juneau. Juneau: U.S. Department of Commerce, NOAA.

Neal, E. G., E. Hood, and K. Smikrud. 2010. Contribution of glacier runoff to freshwater discharge into the Gulf of Alaska. *Geophysical Research Letters* 37: L06404. doi:10.1029/2010gl042385.

O'Neel, S., E. Hood, A. L. Bidlack, S. W. Fleming, M. L. Arimitsu, A. Arendt, E. Burgess, et al. 2015. Icefield-to-ocean linkages across the northern Pacific coastal temperate rainforest ecosystem. *BioScience* 65: 499–512. doi:10.1093/biosci/biv027.

Pasquaud, S., J. Lobry, and P. Elie. 2007. Facing the necessity of describing estuarine ecosystems: a review of food web ecology study techniques. *Hydrobiologia* 588: 159–172. doi:10.1007/s10750-007-0660-3.

Peterson, B. J., and R. W. Howarth. 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. *Limnology and Oceanography* 32: 1195–1213. doi:10.4319/lo.1987.32.6.1195.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. *Annual Reivew of Ecology and Systematics* 28: 289–316.

Scenarios Network for Alaska Planning (SNAP). 2008. Preliminary Report to the Governor's Sub-cabinet on Climate Change. SNRAS Pub. No. MP 2008-06, University of Alaska Fairbanks.

Sklar, F. H., and J. A. Browder. 1998. Coastal environmental impacts brought about by alterations to freshwater flow in the Gulf of Mexico. *Environmental Management* 22: 547–562. doi:10.1007/s002679900127.

Spencer, R. G. M., J. M. E. Ahad, A. Baker, G. L. Cowie, R. Ganeshram, R. C. Upstill-Goddard, and G. Uher. 2007. The estuarine mixing behaviour of peatland derived dissolved organic carbon and its relationship to chromophoric dissolved organic matter in two North Sea estuaries (U.K.). *Estuarine, Coastal and Shelf Science* 74: 131–144. doi:10.1016/j.ecss.2007.03.032.

Tasto, R. N. 1975. *Aspects of the biology of the Pacific staghorn sculpin, Leptocottus armatus Girard, in Anaheim Bay. The Marine Resources of Anaheim Bay.*

Tresierra-Aguilar, A. 1980. Life history of the snake prickleback *Lumpenus sagitta* Willimovsky, 1956. Oregon State University.

Vinagre, C., J. Salgado, H. N. Cabral, and M. J. Costa. 2011. Food web structure and habitat connectivity in fish estuarine nurseries-impact of river flow. *Estuaries and Coasts* 34: 663–674. doi:10.1007/s12237-010-9315-0.

Virginia Department of Game and Inland Fisheries. 2016. Mattaponi River.

West, J. M., G. D. Williams, S. P. Madon, and J. B. Zedler. 2003. Integrating spatial and temporal variability into the analysis of fish food web. *Environmental Biology of Fishes* 67: 297–309.

Wolanski, E., and D. S. McLusky, ed. 2011. *Treatise on Estuarine and Coastal Science*. San Diego: Academic Press.

Appendices

Appendix A: 2013 IACUC Approval Letter

(907) 474-7800
(907) 474-5993 fax
fyiacuc@uaf.edu
www.uaf.edu/iacuc

Institutional Animal Care and Use Committee
909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

July 9, 2013

To: Anne Beaudreau
Principal Investigator

From: University of Alaska Fairbanks IACUC

Re: [465729-2] Food Webs In Flux: Tracking Freshwater Delivery of Nutrients to Coastal Ecosystems

The IACUC reviewed and approved the Response/Follow-Up referenced above by Designated Member Review.

Received:	June 28, 2013
Approval Date:	July 9, 2013
Initial Approval Date:	July 9, 2013
Expiration Date:	July 9, 2014

This action is included on the July 18, 2013 IACUC Agenda.

PI responsibilities:

- *Acquire and maintain all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol and could result in revocation of IACUC approval.*
- *Ensure the protocol is up-to-date and submit modifications to the IACUC when necessary (see form 006 "Significant changes requiring IACUC review" in the IRBNet Forms and Templates)*
- *Inform research personnel that only activities described in the approved IACUC protocol can be performed. Ensure personnel have been appropriately trained to perform their duties.*
- *Be aware of status of other packages in IRBNet; this approval only applies to this package and the documents it contains; it does not imply approval for other revisions or renewals you may have submitted to the IACUC previously.*
- *Ensure animal research personnel are aware of the reporting procedures on the following page.*

(The following information is also available in a printable format in the IRBNet Forms and Templates)

HOW DO I REPORT CONCERNS ABOUT ANIMALS IN A UAF RESEARCH FACILITY?

- All "live" animal concerns related to care and use should be reported to the IACUC
- Email: fyiacuc@uaf.edu Phone: 474-7800
- Report form: www.uaf.edu/iacuc/report-concerns/
- IACUC Committee Members: www.uaf.edu/iacuc/iacuc-info/
- Additional information: www.uaf.edu/ori/responsible-conduct/research-misconduct/ and www.uaf.edu/ori/responsible-conduct/conflict-of-interest/

WHAT SHOULD I DO IF AN ACCIDENT OR INCIDENT OCCURS IN AN UAF ANIMAL FACILITY?

- **For all immediate *human* emergencies call 911** or UAF Dispatch at 474-7721 for less immediate emergencies.
- If you have **suffered an animal bite or other injury**, complete an "Accident/Incident Investigation form" (personal injury) form available at www.uaf.edu/safety/incidentreport-2012.pdf.
- If an accident such as a **chemical spill** occurs, contact the Environmental Health, Safety, and Risk Management (EHS&RM) Supervisor at 474-5617 or the Hazmat Coordinator at 474-7889.

WHO DO I CONTACT IF I FIND A DEAD, INJURED, OR DISTRESSED ANIMAL IN A UAF RESEARCH FACILITY?

- During regular business hours, immediately contact facility staff and/or Veterinary Services Staff at 474-7020.
- After hours or on weekends, immediately contact facility staff and/or Veterinary Services Staff using the contact numbers posted on the "Emergency Contact Information" in the facility or call UAF Dispatch at 474-7721.
- Contact the IACUC at 474-7800 or fyiacuc@uaf.edu if an "Emergency Contact Information" sign is NOT posted in the facility.
- Contact the IACUC if you are not satisfied with the response from Vet Services.

HOW DO I REPORT ANY CONCERNS REGARDING WORK HAZARDS OR ANY GENERAL UNSAFE CONDITIONS?

- Complete an "Unsafe Condition Reporting Program" form, available at the EHS&RM website: www.uaf.edu/safety/unsafe-condition/

WHERE CAN I OBTAIN GENERAL OCCUPATIONAL SAFETY INFORMATION?

- www.uaf.edu/iacuc/occupational-health/

Appendix B: 2014 IACUC Approval Letter

UNIVERSITY OF ALASKA FAIRBANKS

(907) 474-7800
(907) 474-5993 fax
uaf-iacuc@alaska.edu
www.uaf.edu/iacuc

Institutional Animal Care and Use Committee

909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

June 19, 2014

To:	Anne Beaudreau Principal Investigator
From:	University of Alaska Fairbanks IACUC
Re:	[465729-7] Food Webs In Flux: Tracking Freshwater Delivery of Nutrients to Coastal Ecosystems

The IACUC reviewed and approved the Amendment/Modification to the Protocol referenced above by Designated Member Review.

Received:	June 9, 2014
Approval Date:	June 19, 2014
Initial Approval Date:	July 9, 2013
Expiration Date:	July 9, 2015

This action is included on the June 12, 2014 IACUC Agenda.

PI responsibilities:

- *Acquire and maintain all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol and could result in revocation of IACUC approval.*
- *Ensure the protocol is up-to-date and submit modifications to the IACUC when necessary (see form 006 "Significant changes requiring IACUC review" in the IRBNet Forms and Templates)*
- *Inform research personnel that only activities described in the approved IACUC protocol can be performed. Ensure personnel have been appropriately trained to perform their duties.*
- *Be aware of status of other packages in IRBNet; this approval only applies to this package and the documents it contains; it does not imply approval for other revisions or renewals you may have submitted to the IACUC previously.*
- *Ensure animal research personnel are aware of the reporting procedures on the following page.*

(The following information is also available in a printable format in the IRBNet Forms and Templates)

HOW DO I REPORT CONCERNS ABOUT ANIMALS IN A UAF RESEARCH FACILITY?

- All "live" animal concerns related to care and use should be reported to the IACUC
- Email: uaf-iacuc@alaska.edu Phone: 474-7800
- Report form: www.uaf.edu/iacuc/report-concerns/
- IACUC Committee Members: www.uaf.edu/iacuc/iacuc-info/
- Additional information: www.uaf.edu/ori/responsible-conduct/research-misconduct/ and www.uaf.edu/ori/responsible-conduct/conflict-of-interest/

WHAT SHOULD I DO IF AN ACCIDENT OR INCIDENT OCCURS IN AN UAF ANIMAL FACILITY?

- **For all immediate *human* emergencies call 911** or UAF Dispatch at 474-7721 for less immediate emergencies.
- If you have **suffered an animal bite or other injury**, complete an "Accident/Incident Investigation form" (personal injury) form available at www.uaf.edu/safety/incidentreport-2012.pdf.
- If an accident such as a **chemical spill** occurs, contact the Environmental Health, Safety, and Risk Management (EHS&RM) Supervisor at 474-5617 or the Hazmat Coordinator at 474-7889.

WHO DO I CONTACT IF I FIND A DEAD, INJURED, OR DISTRESSED ANIMAL IN A UAF RESEARCH FACILITY?

- During regular business hours, immediately contact facility staff and/or Veterinary Services Staff at 474-7020.
- After hours or on weekends, immediately contact facility staff and/or Veterinary Services Staff using the contact numbers posted on the "Emergency Contact Information" in the facility or call UAF Dispatch at 474-7721.
- Contact the IACUC at 474-7800 or uaf-iacuc@alaska.edu if an "Emergency Contact Information" sign is NOT posted in the facility.
- Contact the IACUC if you are not satisfied with the response from Vet Services.

HOW DO I REPORT ANY CONCERNS REGARDING WORK HAZARDS OR ANY GENERAL UNSAFE CONDITIONS?

- Complete an "Unsafe Condition Reporting Program" form, available at the EHS&RM website: www.uaf.edu/safety/unsafe-condition/

WHERE CAN I OBTAIN GENERAL OCCUPATIONAL SAFETY INFORMATION?

- www.uaf.edu/iacuc/occupational-health/

Appendix C: Approval from non-committee member coauthor to include manuscript in thesis

Douglas Duncan
P.O. Box 211216, Auke Bay, AK 99821
907-359-2892
dhduncan@alaska.edu

May 19th, 2016

Emily Whitney
17101 Point Lena Loop Road, Juneau, AK 99801
ejwhitney@alaska.edu

Dear Emily,

As a coauthor, I give you permission to include the manuscript titled "*Chapter 1: Spatial and temporal variation in the diets of Pacific staghorn sculpin related to hydrological factors in glacially-influenced estuaries*" in your University of Alaska Fairbanks master's thesis.

Sincerely,
Douglas Duncan